STUDIES ON THE GENETICS OF PARAMECIUM.

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TABLE OF CONTENTS.

1. GENERAL INTRODUCTION.

Paramecia belong to the group of organisms known as the Ciliate Protozoa. The Protozoa are the smallest and morphologically the simplest members of the Animal Kingdom. The body of a protozoan is structurally one cell and all the processes necessary for life are carried out within it. In the remaining members of the Animal Kingdom (the Metazoa) the body is composed of many cells and the functions of life are divided between groups of these cells known as organs. Many protozoologists hold the view, first proposed by Dobell (1911), that animals of the protozoan type should correctly be described as a-cellular. The reason for making this distinction is that in a protozoan animal organs of specific function are formed intracellularly, and in higher animals organs are differentiated by the modification or 'organization' of cell-lineages. In protozoa many of the cell organs or 'organellae', such as the gullet of Paramecium or the kinetosome of Trypanosoma, are self-duplicating structures. These organellae perpetuate and maintain themselves in heredity without needing any differentiation process, and indeed if they are lost or removed the animal is unable to replace them. The ciliate protozoa are further distinguishable from other types of protozoans in possessing dimorphic nuclei: a massive macronucleus

controls the normal metabolism of the animal, and the smaller micronuclei are solely concerned in the sexual reproduction of the animal.

However, even allowing these distinctions, the protozoan animal can still be considered as an isolated model of the metazoan cell. The two can be validly compared as nucleo-cytoplasmic systems. The effect of the gene and its mode of action in a nucleo-cytoplasmic system is a problem common to both the study of the protozoan animal and the metazoan cell. Certain aspect of nuclear behaviour, the cytoplasmic organellae, and the detailed life cycles of protozoa, are problems peculiar to protozoological research. Nevertheless the interactions of nuclear, cytoplasmic, and environmental factors in the determination of hereditary characters in protozoa provides information of interest in the study of the functioning of the metazoan cell.

Despite their simple appearance a great deal of morphological diversity exists between different members of the Protozoa, indicating the evolutionary potential of this group of organisms. The variation in the mating behaviour of the species of the genus Paramecium, to be described later, points to a considerable amount of physiological variation between closely similar morphological types. Variation can even be found within the

interbreeding units of protozoa (e.g. the varieties of Paramecium) and at this level it is possible to apply the classical Mendelian methods to the analysis of its hereditary basis.

Paramecia belong taxonomically to the Euciliate Group (Metcalf 1923) of the Class Ciliata (Perty 1852) of the Protozoa (Goldfuss 1817, emend von Siebold 1845). This group of animals retain their cilia throughout the entire life cycle. In paramecia the cilia are distributed evenly over the body surface and the animals therefore belong to the Order Holotrichia (Stein 1859). As the cilia of the gullet are free and do not form an undulating membrane, the paramecia are included in the Sub-order Trichostomata (Butschli 1889, emend Kahl 1930). The Family Parameciidae (Kent 1881) contains all the distinct species of the Genus Paramecium (Hill 1752, emend Stein 1860, emend Wichterman 1953) and one other genus of doubtful status.

Paramecia are free-living, small cigar shaped animals about 100μ-300μ in length, reproducing both by asexual and sexual means. They inhabit areas of water, fresh, brackish, or salt, according to the particular species, and no resistant or encysted stages of the life cycle are known. In the year 1937 Sonneborn discovered the existence of 'mating types' in Paramecium aurelia and as a result

controlled mating and cross-breeding became possible. This has permitted an orthodox Mendelian approach to the study of the heredity of Paramecium, and this organism has subsequently proved to be a valuable material in the study of gene action.

The advantages of the use of Paramecium are that their small size and short generation time allow extensive breeding experiments to be carried out in a short period of time and in a limited space. The genetical study of paramecia in particular offers a more straight-forward analysis of cytoplasmic phenomena and can provide information which is useful in the construction of models for the processes of differentiation in multicellular animals. The question of how the cytoplasmic changes in a single cell-lineage are brought about is an important one in Biology: it is at the same time the problem of early embryonic differentiation and the problem of the complex cytological differentiation of the many different tissues of the adult body.

2. BRIEF REVIEW OF THE LIFE CYCLES OF P. AURELIA AND P. CAUDATUM.

The materials used in this work are the two species of paramecia, P. aurelia (Ehrenberg) and P. caudatum (Ehrenberg). P. caudatum (length 180-300μ) is a larger animal than P.aurelia (length 120-180μ). There is also a difference in the nuclear morphology of the two species: both have one macronucleus, but in P. caudatum there is one large micronucleus and in P.aurelia there are two small vesicular micronuclei.

Paramecia are cultured in units called 'stocks'. A stock is a line of animals descended from one original animal isolated from the wild. A 'clone' is a line of animals descended by asexual multiplication from one individual and it is the culture unit used in genetical experiments. The term 'caryonide' is used to describe a line of animals all containing macronuclei derived from a single macronucleus. The significance of the latter is explained in the account of the reproductive processes of Paramecium given below. This account is based on material described in the reviews of Wichterman (1953) and Wenrich (1954).

Normal asexual multiplication is by binary fission. The animal elongates, constricts around the middle, and divides. The division of the cell is preceded by division of the nuclei. The

micronuclei divide mitotically, and the macronucleus amitotically by a process of elongation, constriction, and separation. The average rate of division at 25° C of P.aurelia (variety 9) is once every eight hours, and of P.caudatum once every ten hours.

Mating can only occur between animals of opposite 'mating type' (see detailed discussion on pp. 12-16). In both species animals of two alternative states or mating types are found. Animals of opposite mating type conjugate when brought together under certain conditions. Normally animals of the same mating type do not conjugate or show mating reaction. After the discovery of the mating types it was revealed that the apparently homogeneous P.aurelia species could be subdivided into 'varieties'. These varieties are the equivalents of subspecies. Each is an interbreeding unit with its two complementary mating types, which do not in general conjugate with any of the mating types of the other varieties. Weak or incomplete mating reactions may occur between animals of different varieties but any resulting offspring are inviable sooner or later.

The first result of mixing animals of opposite mating type is a clumping of the animals into larger groups. Later the animals separate as free-swimming pairs aligned with their gullets facing one another, and meiotic division of the

micronuclei followed by cross-fertilization takes place. The cytological details of conjugation in the two species are different owing to their different number of micronuclei. Description of the cytology of conjugation is due mainly to the work of Hertwig (1889) Maupas (1889), Diller (1936), and Sonneborn (1954b); here the process in P.aurelia will be described first.

When conjugation is achieved the meiotic process begins. Both micronuclei divide twice (meiotically) giving a total of eight haploid products. Seven of these degenerate, and the remaining one retires into the 'paroral cone' - a projecting portion of the mouth region differentiated at conjugation - and the macronucleus begins to split into fragments. The surviving haploid nucleus divides once mitotically forming a 'stationary' and a 'migratory' nucleus. Reciprocal cross-fertilization takes place between the conjugants and a genotypically identical zygote nucleus is formed in each. Shortly afterwards the conjugants separate, and the zygote nucleus in each animal divides twice mitotically giving four products, two of which will differentiate into macronuclei and two will remain micronuclei. The pair destined to be macronuclei swell in size into diffusely staining bodies known as 'anlagen',

and the intensity of staining in these gradually increases until they assume the aspect of mature macronuclei. The fragments of the old macronucleus are lost either by reabsorption or by dilution out in the subsequent divisions. In the first division after conjugation the anlagen are segregated one to each daughter animal, while the micronuclei divide by regular mitosis, thus producing animals with one macronucleus and two micronuclei. From this point the normal asexual division cycle is restored and the animals multiply, maintaining this nuclear number. Since two anlagen are formed after the divisions of the zygote nucleus, the term caryonide has been introduced to describe the line of animals whose macronuclei are descended from one of these anlagen. Thus each exconjugant cell gives rise to a clone which is made up of two caryonides.

The process of conjugation and sexual reproduction of P.caudatum is very similar. In P.caudatum the single micro-nucleus divides by two meiotic divisions to give four products, instead of the eight found at the same stage in P.aurelia. Three of these degenerate and one is left. Fission of this and reciprocal cross-fertilization produce the diploid state again. Macronuclear fragmentation in P.caudatum is delayed until the conjugants have

separated. The zygote nucleus undergoes three fissions giving eight products, four becoming anlagen and four remaining micronuclei. Segregation occurs by two divisions so that each cell again has one macronucleus and one micronucleus. Normal nuclear division begins at this stage and the proportion of one macronucleus to one micronucleus per cell is perpetuated. The difference in nuclear behaviour of the two species at conjugation is important experimentally as the exconjugant divisions produce in P.caudatum four caryonides per exconjugant clone as compared with two in P.aurelia.

In addition to the cross-fertilization process of conjugation there is a self-fertilization process known as autogamy. Autogamy occurs cyclically without heterosexual behaviour or pairing, and has been found in both P.aurelia (Diller 1936) and P. polycaryum (Diller 1954). Whether autogamy occurs in P.caudatum or not is still in doubt (see pages 52-55). It has not been reported in P.bursaria. The nuclear behaviour parallels the sequence observed in the conjugation process, with the difference that there is no cross-fertilization. Instead the 'stationary' and the 'migratory' nuclei of the same animal fuse and reconstitute the diploid phase of the life cycle. Autogamy was described first, from cytological studies, by

Diller (1936). Later his description of the process was confirmed by the genetical experiments of Sonneborn (1947a). One consequence of autogamy is that the genotype becomes completely homozygous. This facilitates the analysis of Mendelian factors, since F1 animals if induced to go through autogamy - experimentally less laborious than outcrossing - will give a 1:1 segregation for any pair of allelomorphs present. The inter-autogamous interval in P.aurelia variety 9 has been found to be of the order of 12-14 days, which is similar to the interval found in variety 1.

In the earlier literature there are many accounts of a process named endomixis which involves the formation of new macronuclei without nuclear fusion, and was first described by Woodruff and Erdmann (1914). It seems possible that the processes described are autogamy. In P.aurelia the genetical evidence of Sonneborn has clearly proved the occurrence of autogamy, and no genetical evidence for endomixis has been obtained.

Conjugating pairs may go through a process known as cytogamy (Wichterman 1939) instead of cross-fertilization. The result is similar to autogamy since haploid nuclei in each conjugant fuse with no reciprocal cross-fertilization. Cytogamy occurs occasionally in P.aurelia, and it is claimed by

Wichterman that cytogamy rather than cross-fertilization is the normal outcome of the conjugation of P. caudatum.

Other processes of nuclear reorganisation have been described, e.g. macronuclear regeneration (Sonneborn 1947a), and hemixis (Diller 1936).

Frequent cross-fertilization or self-fertilization is a necessary part of the life cycle of P.aurelia. If conjugation and autogamy are prevented the phenomenon of 'ageing' appears (see review of Sonneborn 1954a). Aged lines have a reduced division rate, a lowered vitality, and will eventually die. An aged line can be 'rejuvenated' by allowing it to undergo autogamy or conjugation. However beyond a certain point in ageing, conjugation and autogamy fail to save the line and it will become extinct. The basis of the phenomenon, suggested by Faure-Fremiet (1953), is the unbalancing of the genic complement of the macronucleus by its repeated amitotic divisions. The ageing phenomenon has not been encountered in P.caudatum (see p. 54).

3. BRIEF REVIEW OF PREVIOUS GENETICAL RESEARCH WITH PARAMECIUM.

A brief review of previous genetic and related research is given below in order to place the work of this thesis in perspective. The genetical study of Paramecium concerns three systems of inheritance. These systems are the mating types, the 'killer trait', and the antigens. A short account of each follows; the strictly genetical research has been carried out entirely on P. aurelia.

The mating type character has been studied in detail in several ciliates. Mating types have been found in six of the eight species of the genus Paramecium. The character was first discovered in the species P. aurelia by Sonneborn (1937). This species was later found to comprise a system of several sub-species or varieties (Sonneborn 1947a). Each of these varieties, of which nine are known, possesses two complementary mating types. In P. caudatum descriptions of a similar situation have been published. Two mating types were discovered by Giese and Arkoosh (1939) and later Gilman (1939, 1941, 1946-7, 1949, and 1950), Chen (1944) and Hiwatashi (1949), revealed the existence of other varieties each with two complementary mating types. In 1938 Jennings reported the existence of

mating types in P. bursaria, and since then six varieties have been found (Chen 1946). The situation in this species is slightly different, however, since in any one variety a system of multiple interbreeding mating types may be found. Animals of one mating type will mate with animals of any other mating type of the same variety, but not with animals of their own mating type. The other species of Paramecium are less well studied, but the situation in each corresponds either to the P. aurelia or the P. bursaria system.

Diverse mating types are probably a general character of the ciliates, since several species of different genera - Euplotes patella (Kimball 1943), E. happa (Katashima 1952), Oxytrichia bifaria (Siegel unpublished, referred to by Nanney and Caughey 1953), Stylonichia putrina (Downs 1952), and Tetrahymena pyriformis (Elliot and Gruchy 1952) - are now known to possess mating types. In each case the general organization of the breeding system has characteristic features. For instance in T. pyriformis, according to Nanney and Caughey (1953), there is a multiple mating system involving seven mating types, any one of which will mate with another but not with itself. In E. patella Kimball discovered a similar situation involving six mating types determined by a system

of three pairs of allelomorphic genes, each controlling one diffusible conjugation inducing substance: the presence of all three complementary substances being necessary for conjugation to occur.

(It is possible also that each species of the genus Paramecium has its own characteristic mating type system, since the work described on pages 35-41 does not confirm the claim that the mating type system in P. caudatum is similar to that of P. aurelia.).

The inheritance of the mating types of paramecia has been studied in detail in P. aurelia (Sonneborn 1947a). Among the nine varieties of P. aurelia two systems of mating type inheritance are found. In varieties 1, 3, 5, and 9, a system of 'caryonidal' inheritance is found. In these varieties animals of any one caryonide possess the same mating type which is inherited unchanged through the asexual divisions. After conjugation or autogamy the mating type may change. Sonneborn found that the mating type is redetermined when the new anlagen are differentiated after the meiotic divisions and karyogamy. Each caryonide arising possess one mating type only which is inherited unchanged until the next conjugation or autogamy. The mating types are redetermined in a random manner; sister caryonides may be of the same mating type or of

complementary mating types. This type of inheritance is peculiar to these varieties and is distinctive in that it is independent of any genic segregation since it occurs regularly in homozygous lines.

Notwithstanding this the first Mendelian character to be demonstrated in Paramecium was the recessive gene mt in variety 1. Certain variety 1 stocks displayed only one mating type, the complementary type never appearing even after the formation of new macronuclear elements. The crossing of such a stock to the normal 'two-type' stock produced a segregation showing that the 'one-type' character was determined by the recessive allele mt, and the 'two-type' character by the dominant allele Mt.

The second system of inheritance involves varieties 2, 4, 6, and 8. The mating types of the exconjugant clones are generally the same as those of their cytoplasmic parents. The mating types are inherited unchanged during asexual multiplication and through autogamy. This cytoplasmic type of inheritance resembles the 'maternal' inheritance of characters in higher organisms.

At the present time the theoretical basis of the mating type system is not clear. The mating types provide an interesting example of the

assortment and maintenance of non-genic determinants of heredity. However it is dependent on the existence of a macronucleus and consequently cannot be made directly analogous to anything outside the ciliate protozoa.

The inheritance of the character known as the 'killer trait' has also been studied in detail and concerns varieties 2, 4, and 8, of P. aurelia. In varieties 2 and 4 it was observed that certain stocks ('killers') were able to cause the deaths of certain other stocks ('sensitives') (Sonneborn 1938). The agent of killing is a poisonous substance - paramecin - secreted into the culture fluid by the killer animals. One particle of this substance is sufficient to cause the death of a sensitive animal (Austin 1948). Chemically the particles are thought to contain DNA (Van Wagtendonk 1948). Preer (1948) discovered that every killer stock carried in its cytoplasm quantities of Feulgen positive particles. The presence of the particles was correlated with the secretion of paramecin and provided immunity against its toxic effects. A gene component of the system was found by Sonneborn (1943) who noted that a dominant allele was required at a particular locus to maintain 'kappa' (the Feulgen positive particles)

in the cytoplasm. Animals homozygous for a recessive gene at this locus are unable to keep the kappa particles in their cytoplasm and therefore are permanently sensitive. An animal of the dominant genotype which loses its kappa particles will also be sensitive since the kappa particles are self-reproducing particles and do not arise 'de novo'.

Various qualitative differences in the killing action are observed in different stocks and are inherited cytoplasmically, indicating that different types of kappa exist. Dippell (1950) has analysed four different killing types, which had arisen by mutation in one stock, and she has shown that the differences are in the cytoplasmic factors and not due to the mutation of genes in the nucleus. Morphological and serological differences have also been found between the kappa types. Certain stocks derived from original killer stocks carry particles (named 'pi') which produce no phenotypic effects, i.e. no killing action and no immunity (Hanson 1954).

The three known killers of variety 8 are distinctive (Siegel 1953). They carry cytoplasmic particles ('mu'), but no secretion is detectible and a peculiar phenomenon of mate killing is found. Killer animals cause the death within a few fissions of any sensitive animal with which they conjugate. Mate killers are not known outside of variety 8.

These facts are of interest in various fields of biology. The kappa particles have been compared to virus particles; like the viruses they are self-reproducing particles within a cell, but unlike them they are not infectious. They have also been invoked as evidence for the support of plasmagene theories of development and differentiation. The interpretation of kappa as a virus implies that it is a body of extraneous origin, while the plasmagene interpretation implies that the kappa particles are integral extra-nuclear units of the animal's normal heredity. The kappa particles are the only satisfactory examples of the plasmagenes which are envisaged by Darlington and others (Darlington and Mather 1939) as being of universal occurrence and a part of the hereditary make-up of an organism located in the cytoplasm. However, the fact that some paramecia have no kappa counts against this interpretation.

It is best to regard kappa as a specific type of self-reproducing particle found in certain stocks of P. aurelia which has properties placing it intermediate between organisms of the virus type and hypothetical hereditary particles borne in the cytoplasm. Leaving aside questions of the nature and origin of kappa particles, there remains the interesting genetic relationship that exists between

the nuclear genotype and the phenotypic units in the cytoplasm.

The third system of inheritance studied in detail in Paramecium concerns the antigenic properties of the organism. This work has been reviewed fully by Beale (1954), and only the main points are given below. Antiserum is prepared by injecting paramecia into a rabbit. The effect of the antiserum obtained is measured by its ability to immobilize living paramecia, of the type injected.

The antigens are a convenient trait for genetic analysis since paramecia display a great deal of antigenic variation. Each stock has a number of different antigenic types, only one of which is expressed at any one time. The transformation from one type to another can be brought about by change of the environmental conditions or by treatment with homologous antiserum. This transformation from one type to another occurs rapidly and no stable intermediate states between any two antigenic types are found. It is usual to denote these antigenic types of one stock by letters - A, B, C, etc. In variety 4 of P. aurelia as many as eight serologically distinct types have been found in the one stock. In some cases the antigenic types expressed by different stocks are serologically similar and in

other cases they are distinct.

By crossing different stocks which can be serologically marked, Mendelian analysis can be carried out. This was done first by Sonneborn (1950a), who mated the A antigenic types of stocks 51 and 29 of variety 4 which are serologically similar yet capable of being distinguished by their cross-reaction with a third non-homologous antiserum. The antigenic types 51A and 29A segregated in Mendelian proportions as would be expected if they were determined by a pair of allelomorphic genes. Therefore the type 51A is said to be controlled by a gene $\underline{a}^{51}$ and the type 29A by a gene $\underline{a}^{29}$. Later Sonneborn found that the H antigenic types of stocks 51 and 29 are controlled by another pair of genes, $\underline{h}^{51}$ and $\underline{h}^{29}$ respectively, at a different locus from the $\underline{a}$ alleles. The two loci are not linked.

In variety 1 a similar situation has been analysed by Beale (1952). Here normally three antigenic types S, G, and D, are found in each stock. The S antigenic types in each stock are controlled by the allelomorphs of one gene, the G types by a second gene, and the D types by a third gene. The three genes are unlinked. An effect of the environment was observed in that the S types

predominate during growth at low temperatures, the G types at intermediate temperatures, and the D types at high temperatures. Each allele however besides controlling the specificity of the antigenic type, also controls its temperature stability; e.g. all G_x types are normally stable between 18° C and 27° C, but each individual G type has its characteristic range within these limits.

The maintenance of a particular antigenic type is thus dependent on the nuclear genes, the constitution of the cytoplasm, and the environment. A particular antigen-determining gene is allowed expression only if a certain cytoplasmic state is present, and the latter is dependent for its existence on the environmental conditions prevailing.

The antigen situation can be used as a model of a cellular system of a general type in which diverse types of cell can arise in a cell-lineage and then multiply unchanged (Sonneborn 1947b, Beale 1954). The action of the environment (e.g. the temperature effect in variety 1) is similar to the action of the 'organizer' in metazoan development and the characteristics of each cytoplasmic state (e.g. the response of each cytoplasmic type to a given temperature) resembles the phenomenon of 'competance' of an embryonic

tissue to be affected by an organizing substance. The point of most interest about this model is that no genic changes or mutations are required to explain the appearance of a number of different phenotypes which can remain distinct for many cell generations although still carrying the same genotype.

The P. aurelia antigen system has also aroused interest in that it shows similarities to the 'relapse' strain phenomenon of pathogenic trypanosomes and other organisms. After treatment of an infected animal with a trypaniscidal drug a subsequent fever or relapse may occur. When the original and the relapse strain or strains are compared they are often found to be quite distinct serologically. These relapsed antigenic types are quite stable and can be maintained by serial passage through the host species. In the most recent work (Inoki et alia 1952 a and b) on Trypanosoma gambiense, it has been shown that relapse strains can revert to the original type and that the transformation from one strain type to another can be accomplished experimentally by treatment of isolated trypanosomes with homologous antiserum. It is also found that, like Paramecium, serologically the intra-strain antigenic types are more distinct than are the differences between strains considered

distinct species. Genetic analysis of trypanosome strains is not possible yet as no sexual stages are known, but drawing on the knowledge obtained from the genetics of P. aurelia, an analogous explanation of the existence and the mechanism of transformation of the relapse strains can be proposed, which has the attraction of not requiring either the selection of pre-existing mutations or the induction of new ones.

This brings to an end the short account of the existing knowledge of the genetics of Paramecium. In this thesis work on P. caudatum and variety 9 of P. aurelia is described.

An attempt has been made to study the genetics of P. caudatum. This species is of common occurrence and has been used in biological research for a long time but no genetical studies have been made on it. Consequently a comparison of the genetics of this species to the situation already known in P. aurelia would be profitable and how much the difference in morphology is reflected in the genetical system would be seen.

In P. aurelia a new variety (variety 9) found very conveniently in Scotland has been studied. The character used for study is the antigenic variation. The variation and the genetic control of the antigens of variety 9 has been compared to the situations already known in varieties 1 and 4.

The experimental work is consequently described and discussed in two sections and a short introduction before each relates the problems tackled to the research described above.

4. METHODS.

(i) The Method of Culture.

The methods used for the culture and breeding of Paramecium have been fully described by Sonneborn (1950b). In the present work the methods employed have followed closely those of Sonneborn. The following is an outline of the technique.

The animals are cultured in a medium made by the infusion of dried powdered lettuce in glass distilled water. The lettuce medium plus a trace of $CaCO_3$ is sterilized by autoclaving. Before use the sterile medium is inoculated with a strain of Aerobacter aerogenes - maintained on agar slants - and incubated for twenty-four hours at 35°C. If necessary the pH is adjusted to approximately 7, using $Ca(OH)_2$.

Mass cultures are normally carried in test-tubes partially filled with medium. Larger cultures, for example for injection, are grown in conical flasks. Animals are isolated singly by hand under the binocular microscope with a micropipette and are grown in drops of medium set in depression slides.

Such methods are satisfactory for normal asexually multiplying cultures. To induce sexual behaviour under controlled conditions special methods are required. Normally in P. aurelia

sexual behaviour results if test-tube cultures are grown rapidly to a high density of animals and subsequently allowed to starve slowly. If samples are mixed at intervals, a period will occur during the starvation procedure when the animals will show sexual behaviour and will conjugate, provided of course the samples mixed are of opposite mating type.

The procedure for inducing the onset of autogamy is to continue a culture by daily isolation in depression slides, allowing each daily isolate to multiply exhausting the medium thereby starving itself. Autogamy should occur after a certain number of divisions from the previous autogamy. Animals, having undergone the requisite quota of divisions during serial culture, will be ready to undergo autogamy and such animals will be found in the starved depressions of this age. The presence of autogamy is detected by temporary microscope preparations stained with aceto-carmine. The fragmentation of the macronucleus, which takes place during autogamy, is easily seen in these preparations.

(ii) The Difficulties encountered in the Culture of P. caudatum.

The standard methods for the induction of sexual behaviour and autogamy used with P. aurelia do not unfortunately work with the related species

P. caudatum. The starvation method succeeds spasmodically in bringing about conjugation, but it is not consistently repeatable. Some stocks of P. caudatum will remain strongly reactive over considerable periods extending up to several months, while others become only transiently reactive or do not become reactive at all. Why this should be so has not been apparent. Gross alterations of the concentration of the culture medium, pH, oxygenation, and the type of bacteria, have produced no positive effect. Mating reactivity does not seem to be directly related to the degree of starvation, as reactive animals may be slender or plump. The addition of dried yeast to the culture tubes produced better reactions, probably however as a result of the higher density of growth obtained. No evidence of any diurnal cycle in mating reactivity, as was reported by Maupas (1889), has been observed.

The methods used by previous workers to induce conjugation in P. caudatum are diverse and contradictory, many however were hampered by a lack of knowledge of the existence of mating types. Hopkins (1921) found his stocks conjugated best if well fed, while Maupas (1889) and others following his methods obtained conjugation by abrupt starvation. Maupas found this treatment of mass

cultures only occasionally effective and noted that other factors must be involved (one of which of course may have been the absence of diverse mating types). Gilman (1941), Chen (1944), Hiwatashi (1949), and Giese and Arkoosh (1941), used a like procedure, various refinements like centrifugation and suspension in distilled water being included. Zweibaum (1912) found that the addition of salts in low concentration (particularly halogen salts of low molecular weight) produced high intensities of conjugation. Jollos (1921) and Hopkins (1921) were unable to confirm this, and Ball (1925) found that the only stock in his material which responded, conjugated equally well whether placed in one of Zweibaum's solutions, distilled water, or fresh culture fluid. Barbarin (1938) found conjugation occurred on passing hydrogen through the culture (a procedure designed to produce asphyxiation). These cultures had not conjugated when starved. Barbarin attributed this to the different balance of the metabolism of fat and glycogen produced under these conditions. However Gause and Smaragdova (1939) did not find this an effective method of inducing conjugation.

The contradictory nature of the evidence suggests that the methods used are only incidentally concerned with the mating reactivity and that an

effective process of induction has yet to be found. Lack of knowledge about the physiological nature of the reactive state and hence of its induction has prevented all but the most empirical approaches to a solution of the problem.

The lack of a method to produce mating presented a barrier to genetic analysis, and eventually proved to be an insurmountable difficulty. F1 clones could be extracted in various experiments, but very few of these ever became sexually reactive. The lack of F2 clones, along with the absence of any process corresponding to the autogamy of P. aurelia, has prevented Mendelian analysis of the characters studied.

(iii) The Serological Methods.

For the production of antibodies a homogenate of paramecia of uniform antigenic type is injected intravenously into a rabbit.

Approximately 250 c.c. of culture fluid are filtered through cotton wool to remove the debris, and the filtrate is concentrated by Berkfeld filtration and centrifugation. When a reactive serum is available from previous work, the material for injection is checked and rejected if it is not more than 90% pure for the antigenic type in question. The concentrated paramecia are

homogenised by forcing them through a syringe needle several times. One c.c. of homogenate is injected into the marginal vein of the ear of a rabbit; five similar injections are repeated over the course of three weeks. The animal is bled after a lapse of 10-12 days from the last injection, and the serum is separated, frozen, and stored in two c.c. units. Before use the complement fraction (which produces a non-specific effect on paramecia) is inactivated by heat. The serum is diluted to a convenient strength with distilled water. In some cases the titre of the serum falls rapidly after the first bleeding and a 'boosting injection' is required before a further bleeding.

The method of titrating the serum is to measure the time taken for a living paramecium to become immobilized in a standard dilution of the anti-serum. A dilution of 1/50 is used normally and the animals are observed in the antiserum up to a limiting time of two hours.

In January 1955 all stock rabbits were inoculated against myxomatosis virus by injection of shope fibroma virus (Wellcombe Brand). One test rabbit was inoculated and bled after three weeks, and the animal was then used to produce an antibody

against a paramecium antigenic type - 531HT. The 'blank serum' (the anti-shope fibroma serum) and the anti-531HT serum were then tested. The blank serum had no effect on paramecia of 531HT and various other antigenic types, even at a dilution of 1/5. The anti-531HT serum was obtained at normal strength specific against animals of type 531HT. It is therefore concluded that inoculation with myxomatosis vaccine does not interfere with the production or the specificity of the paramecia-immobilizing antibodies.

(iv) The Methods used in Collecting.

Samples of pond and other fresh waters were brought to the laboratory and examined. Samples are usually taken from still water with a fair amount of decaying vegetation and water plants present. Collections are made in small glass tubes, several from each locality. After collection a quantity of bacteria-lettuce medium is added to each tube and the tubes are incubated at 25°C for 24 hours. The tubes are then inspected and any Paramecia found isolated. Inspection of the tubes is repeated again twice at two day intervals.

(v) List of stocks.

All the materials used in this work were collected from the wild in the course of the experiments.

Stock Designation.	Date	Locality of Collection.
(a) P.caudatum.		
1a	Sept. '52	Union Canal, Winchburgh, nr. Edinburgh.
1b		" " "
2		Union Canal, Philipstoun, nr. Linlithgow.
3		Linlithgow loch.
5		Blackford Pond.
(Series X1-X50)	Sept. '53	" "
7	Sept. '52	Carbeth Loch, nr.Glasgow.
10		Union Canal at Linlithgow.
15		Union Canal at Linlithgow Quarry.
18		Loch Cote nr. Edinburgh.
20		River Avon nr. Edinburgh.
21		Pallanza, Italy (Collected by Dr. G. H. Beale).
22		Bayfordsbury, England (coll. G.H.B.)
24	Oct. '52	Threipmuir Reservoir, Edinburgh.
28		Water of Leith, Balerno.
31		Gogar Burn, nr. Edinburgh.
32		Union Canal at Hermiston.
33		"
35		Bardowie Loch nr. Glasgow.
39		Dunsappie Loch Edinburgh.
42		St. Margt's Loch, "
43		Duddingston Loch, "
46		Figgate Pond, "
47		Liberton Water Works, Edinburgh.
60	Aug. '53	River Seine, Paris (coll. G.H.B.)
61		River Seine, Paris. (")
67		Chantilly nr. Paris (coll. G.H.B.)
68		Chantilly (")
69		River Seine, Paris (coll. G.H.B.)
70	Sept. '53	Burton-on-Trent, England (coll. G.H.B.)
J1) J2) J3) J7) J8) J15)	- '52	Newcastle England (recd. from Mrs. Johnstone, Kings College, Newcastle)

Stock Designation	Date	Locality of Collection.
(b) P. aurelia.		
503/1-2	Sept. '52	St. Margaret's Loch, Edinburgh.
504		Figgate Pond, Edinburgh.
505		Strathblane Pond, nr. Glasgow.
506		Union Canal, Kingsknowe, Edinburgh.
509		Union Canal, Ratho Park, nr. Edinburgh.
510/1-80	Sept. '52 Jan. '55	Blackford Pond, Edinburgh. "
518	Aug. '53	Chantilly, nr. Paris (coll. G.H.B.)
530	Sept. '54	Blackford Pond, Edinburgh.
531		"
532	Aug. '54	Cernay-la-Ville, nr. Paris (coll. G.H.B.)
533/1-2		Gif Pond, Gif, nr. Paris (coll. G.H.B.)
534/1-9		La Fillolière, nr. Cernay, France (coll. G.H.B.)

The P. caudatum material was identified visually and later permanent microscope preparations (Feulgen stained) were made in order to see the nuclear morphology and confirm the species classification.

The P. aurelia material was similarly identified by visual inspection and later tested for the variety by mating reaction.

5a - EXPERIMENTAL WORK WITH P. CAUDATUM.

(i) INTRODUCTION.

It was planned to make a study of the genetics of P. caudatum. Till now all genetic research has concerned the species P. aurelia, and therefore it was decided to begin a parallel series of studies on another species, and in particular to make a complementary investigation of its antigenic variation, eventually leading to a serological comparison of the two species.

In Scotland P. caudatum is an extremely abundant protozoan, found in almost any body of fresh water. This attribute makes the species a convenient object for a detailed study of the geographical and ecological distribution of the different antigenic types.

Further since this species is a relatively large form - about 250μ in length - it has been preferentially selected for use in a wide variety of zoological and physiological work. It was used extensively by Jollos (1921) in his work on the phenomenon of 'dauermodification', and it would be desirable to repeat such work using modern genetical techniques.

It has been found however that this species is not as amenable to laboratory culture

conditions as P. aurelia. In addition the behaviour of the stocks collected in Europe does not correspond to the published accounts of the behaviour of material collected on the American continent and in Asia.

Consequently the body of this part of the thesis is taken up with questions of the general biology of the species and the differences from rather than the similarities to the situation in P. aurelia.

(ii) THE BREEDING SYSTEM OF P. CAUDATUM.

(a) The Mating Reaction. In order to provide a basis for a genetical approach to the study of this species a number of stocks were extracted by single isolations from samples collected in a variety of localities. The thirty-five stocks listed on pages 32 and 33 were isolated; twenty-one come from places in the vicinity of Edinburgh and Glasgow, eight are from three places in England, five are from France, and one from Italy.

On mixing reactive clones of these stocks it was possible to place all thirty-five in one 'variety', using the standard criterion of sexual reaction and conjugant formation. This was a rather unexpected finding since the latest assessment of Gilman (1950) provides for the existence of thirteen varieties. In Japan Hiwatashi (1949) was

able to distinguish four varieties among seventeen collections, two of which correspond with two of the American varieties and two of which are new (Gilman 1950). (Chen (1944) obtained three varieties out of forty-one clones from three collections made in China. In addition he claims to have isolated a fourth variety from the exconjugants of one of these varieties. However as he did not in his experiments take sufficient precaution to prevent and detect selfing, the claim is placed in doubt).

In addition to the series of stocks from different localities eighteen reactive stocks were obtained from the one area of water - Blackford Pond, Edinburgh. All eighteen could similarly be included in the solitary variety so far discovered.

A selection of fourteen of these European stocks was sent to Dr. L. Gilman in the University of Florida so that he might compare them against his American variety type-cultures. These stocks were later determined by him as belonging to the variety 2 complex of his system. The variety 2 complex is a group of five varieties which are related in that they show varying amounts of cross-reaction with one another (Gilman 1949). He has to the present not been able to find any correspondence between the stocks received and the individual varieties of the variety 2 complex.

Further he was inclined to regard each one of these fourteen stocks as representing a new variety, because each produced a different spectrum of cross-reactions with the established members of the variety 2 complex (private communication).

In this laboratory using the same criterion for delineating varieties as used by Gilman, namely mating reaction and the ability to form conjugants, none of the thirty-five European stocks could be grouped into sub-varietal units. In fact it was noted that the variation in the results of mating two stocks at different times was of the same order as that obtained by Gilman in the reactions between the different members of the variety 2 complex: e.g. stock 7 crossed with stock 32 gave very strong mating reactions but failed to form conjugants, while at a later mating a considerable proportion of conjugants were formed. It therefore does not seem wise to attempt to construct a system of varieties on the basis of the mating reaction alone.

None of the American type stocks have been received and their reactions with the residue of the European collection is not known.

(b) The Viability of Stocks after Mating. It was noted from the beginning that exconjugants

TABLE I. - THE VIABILITY OF THE OFFSPRING FROM CROSSES OF DIFFERENT STOCKS OF P. CAUDATUM.

STOCKS - MATING TYPE II.	STOCKS - MATING TYPE I.													
	X2	X8	X11	X29	X31	X26	46	1A	24	43	J8	18	67	28
X6	100		66	66	80									
X22	100		80			100		90						25
X38	90		25	66	80	100	90	50						5
24	33	0	0	0	50	50	5	10-40		50	90	30	0	0
1A					100			100						
22									0		90			
5								0				50		
35										0				
28					100			0						

Figures are the %age of exconjugant clones surviving. In all cases at least 12 pairs of conjugants were observed and the %ages were calculated from a minimum of 24 caryonides isolated from these pairs.

obtained by mating diverse clones were often inviable. In some cases all the exconjugant lines died, in others there was death of a certain proportion of the lines, characteristic for the particular cross made. Many deformed and improperly disjoined animals in chains were observed. (The cytological analysis of the situation is described separately on pages 41-47). This situation is markedly different from that observed in crosses within a variety of P. aurelia. Here the F1 clones are usually normal and inviability appears only in the F2 or backcross generation, as would be expected on the assumption of the existence of recessive lethal combinations of genes. The mortality of the F1 clones of P. caudatum indicates the existence of some barrier to mating, and, perhaps that the European material could be split into sub-varietal units on these grounds. The outcome of mating diverse stocks is set out in Table I. The large number of blank squares results from the difficulty of inducing any two stocks to mate. It was impossible to cross stocks by design, and therefore these were merely crossed as they became reactive with any other stock which happened to be reactive at that time.

After the finding of inviability following

crossing of different stocks, a large number of stocks were isolated from one habitat (Blackford Pond) to discover the effect of mating animals found within one geographical unit. In Table I the results show that these Blackford Pond stocks (those prefixed X) give offspring of high viability when crossed among themselves, but in crosses between stocks of different geographical origin varying degrees of inviability of the offspring are found.

However owing to the meagre nature of the data only very general conclusions can be drawn; namely (a) that the amount or lack of viability in a particular cross is a characteristic value for that cross and repeatable obtained, and (b) that crossing animals from the same geographical unit (Blackford Pond) always produced good or complete viability, while crossing stocks of diverse origin gave results extending from non-viability to complete viability.

Evidence such as this tends to indicate that in the P. caudatum material there is a system of mating compatibility operating between stocks which varies in a continuous manner from complete to non-compatibility. The inclusion of F1 viability as an extra criterion in the delineation of varieties does

not make varietal differentiation in this species any more obvious.

(iii) THE CYTOLOGICAL DETAILS OF CONJUGATION.

The process of conjugation has been studied cytologically. The method used was a modified Feulgen procedure with fixation in Schaudinn's fluid. Animals were allowed to mate and then stained in lots of about twenty pairs at carefully timed intervals after the initiation of conjugation. A cross between two stocks(1A and 24) giving offspring of low viability, was compared with a cross of two Blackford Pond stocks (X2 and X22) which produce exconjugants of high viability.

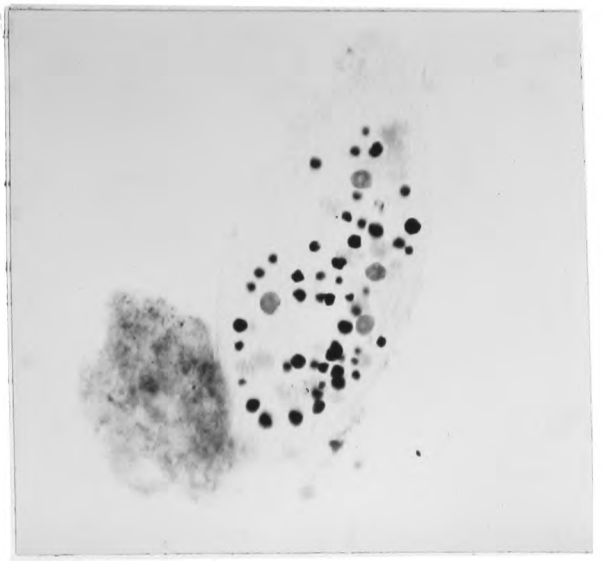
Nuclear events appear quite normal during the meiotic divisions of the micronucleus. Stages corresponding to those described by Hertwig (1889) and subsequent authors were observed. An estimate of the chromosome number was not possible owing to the small size and close arrangement of the chromosomes, and to the lack of flat metaphase plates as the division spindles are invariably orientated in the long axis of the cell.

Differences in chromosome bulk have been used by Diller (1940) and Chen (1940) (with P. bursaria) as an index of polyploidy. No such differences, which might also have explained the F1

inviability, were observed in this material. Dippell (1955) working with stocks of variety 4 of P. aurelia has been able to show aneuploid differences in stocks which when crossed give an F2 of very low viability. Differences of this scale between the different stocks of P. caudatum would not have been detected by the staining method used here.

Abnormalities are first observed at the period, after the animals have separated, when the old macronucleus fragments and the four new anlagen appear. A comparison of the normal (1 - 4) and the abnormal (5 - 11) anlagen differentiation is shown in the accompanying microphotographs. The anlagen developed are misshapen in form (photographs 8, 10 and 11) and abnormal in number (ph. 9), or often not visible at all (ph. 6). The fragmented old macronucleus may not be reabsorbed (ph. 5), or, hypertrophy of one or more of the fragments may occur (ph. 7), even in the presence of differentiating anlagen (ph. 9).

Diller (1940) has figured examples of exconjugants with abnormalities regarding the number and the appearance of the anlagen, but the subsequent viability of the animals was not known. Also in two exconjugant animals from the low

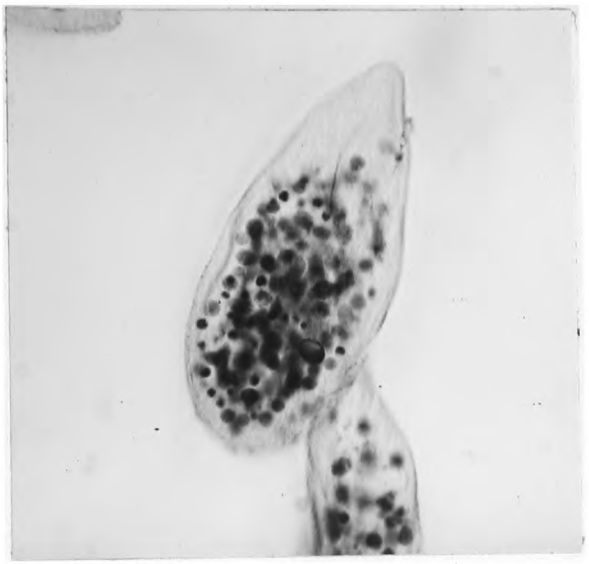


1. Normal cross - exconjugant. Four anlagen. (X300).

2. Normal cross - exconjugant. Four anlagen at later stage. (X300).

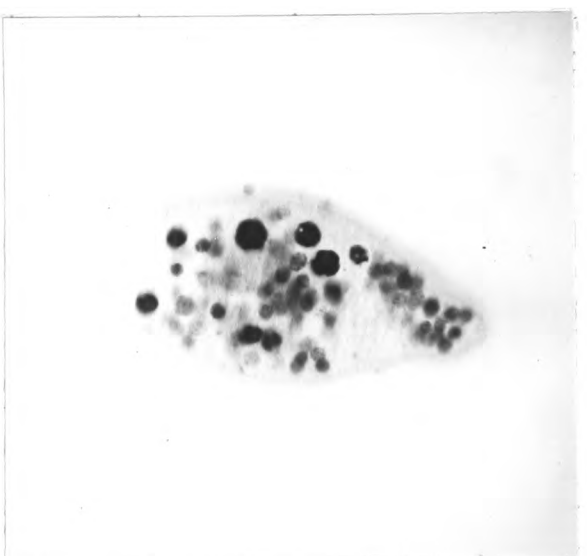
3. Normal cross - after first division. Two anlagen. (X300)

4. Normal cross - after second division. One anlagen. (X300).



5. Abnormal cross - exconjugant having failed to divide. No anlagen visible, excess of fragments. (X300).

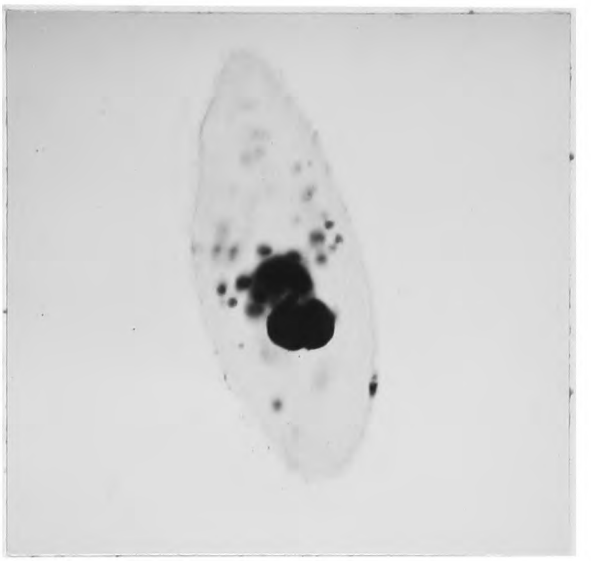
6. Abnormal cross - exconjugant having failed to divide. No anlagen. (X300).



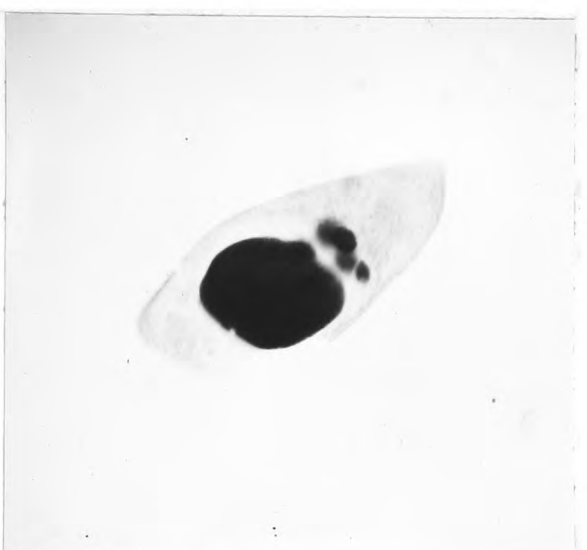
7. Abnormal cross - exconjugant having failed to divide. Faint anlagen, hypertrophied fragments. (X300).

8. Abnormal cross - exconjugant having failed to divide. Four anlagen of diverse appearance. (X300).

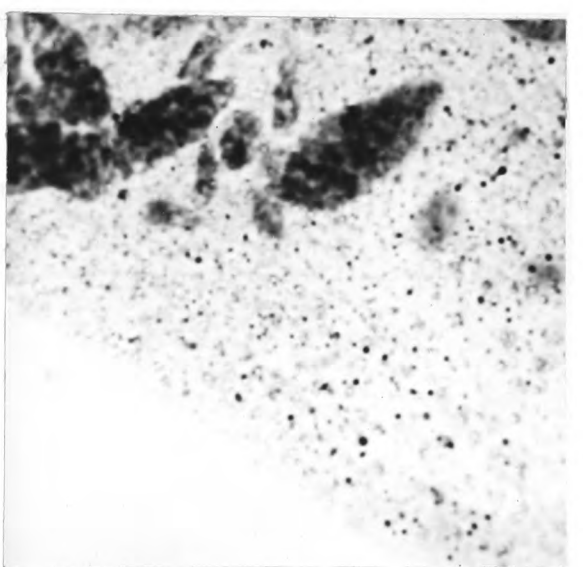
9. Abnormal cross - after first division. One large misshapen anlagen and hypertrophied fragments. (X300).



10. Abnormal cross - after second division. Large misshapen macronucleus. (X300).



11. Abnormal cross - after second division. Hypertrophied macronucleus. (X300).



12. Abnormal cross - after first division. Cytoplasmic particles. (X1800).

viability cross a large number of Feulgen positive particles were present in the cytoplasm comparable in size to kappa particles. These were taken to represent merely one aspect of the abnormal macronuclear behaviour (ph.12). The two animals concerned were exconjugants which had failed to go through the first post-conjugation fission; no comparable cells were found on any of the other slides or on the same slide.

This period of visible nuclear abnormality is also the time of death of the cells. Animals rarely complete both post-conjugation fissions, those which fail to divide often increase greatly in size and can remain alive for as long as fifteen days at 25°C. The time of appearance of the abnormalities may mean that a system of nucleo-cytoplasmic incompatibility is operating, such that new anlagen of mixed genotype fail to develop in a foreign cytoplasm.

Observations were therefore made on conjugating animals and the time of persistence of the 'cytoplasmic bridge' recorded as a measure of cytoplasmic exchange. The cytoplasmic bridge is the fused region of the pellicle through which nuclear cross-fertilization occurs. It is the last region of the conjugants to separate and is visible as a bridge between the conjugants for

varying periods of time. A cross of two stocks (X2 and 24), giving about 33% viable offspring, was observed and the duration of the bridges and the subsequent history of the clones recorded. The time of cytoplasmic bridge connection varied from 30 seconds to 30 minutes with an average period of 4.5 minutes, but no correlation between the time of bridge persistence and subsequent viability was obtained. It is not of course known whether an amount of cytoplasm sufficient to affect the situation could be transferred during these periods.

It was noted that all eight isogenic caryonides produced from one conjugating pair are not equally viable. The surviving lines arise in a random manner, indicating that the abnormalities are not solely nuclear in origin.

The situation in P. caudatum bears some resemblance to the mate killing phenomenon of variety 8 of P. aurelia described on page 17 In the latter nuclear damage is associated with the death of the sensitive clones. In the P. caudatum material however the presence of Feulgen positive particles was not detected.

The evidence tends to indicate that a nucleo-cytoplasmic incompatibility system exists, but owing to the absence of experimental proof the possibility of other explanations cannot be excluded.

(iv) THE MATING TYPES.

It is now necessary to consider the mating types themselves in more detail. In P. caudatum, as will be described later, there does not appear to be any regular nuclear reorganization process comparable to autogamy. Consequently the mating types remain constant during long periods of vegetative culture.

The study of the inheritance of the mating types after the meiotic process was not possible owing to the extreme difficulty of obtaining F1 reactive clones. In all only fourteen exconjugant clones were obtained which were both reactive and antigenically marked with respect to their cytoplasmic origin. The only information which can be gleaned from these meagre results is that both mating types can be extracted after conjugation, and that there is a tendency for the mating type of the exconjugant clone to be the same as that of its cytoplasmic parent. Thirteen of the fourteen clones were of the same type as their parent, while one had changed to the opposite type. (Chen(1944) claims to have found the existence of a caryonidal form of mating type inheritance in his Chinese stocks of P. caudatum, but the fact that he segregated the exconjugant pairs to four instead of the eight clones required

to isolate caryonides makes the claim anomalous).

The mating type of each stock of P. caudatum was found to remain unchanged for long periods (up to at least three months). However changes of mating type and selfing cultures were observed occasionally. Giese and Arkoosh (1939) found that one of the mating types in their material changed type, presumably in their view, as the result of autogamy in the culture. Gilman (1939) noted changes of mating type in his material. He found that one mating type reproduced true to type much longer than the opposite type. But whether the mating type changes coincided with a period of nuclear reorganization was not known.

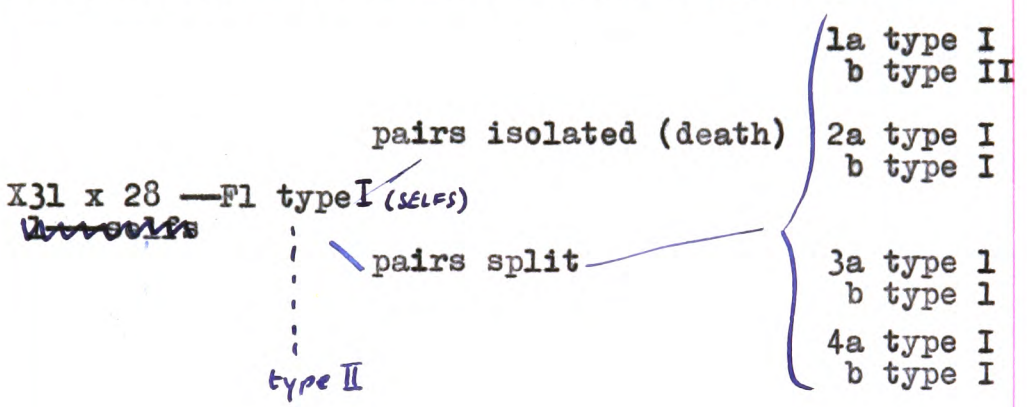
In selfing cultures of the European material on three occasions clones of opposite mating type have been obtained by the separation of animals in the early stages of conjugation. These selfing lines were from stocks recently isolated from the wild. The appearance of two mating types within one line may be an indication of the occurrence of a preceding autogamy, but no cytological observations were made which could have detected this.

Another more prevalent mating type change was noted which is not comparable to any of the established kinds of mating type change in

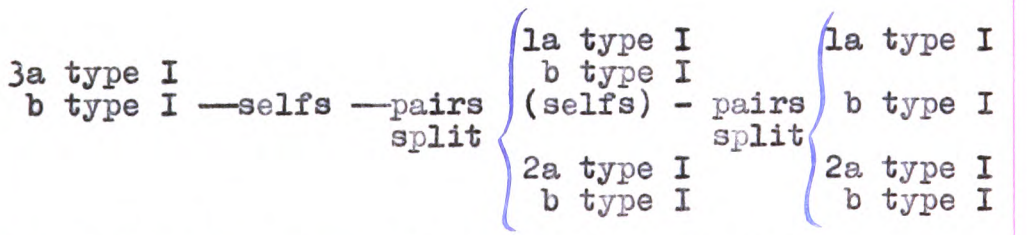
paramecium studies. Particularly in stocks maintained in a reactive state over a long period, it sometimes happens that the whole clone appears to switch mating type from one type to the opposite; i.e. a culture previously reacting as type I now reacts entirely as Type II when compared against a series of other stocks. These observations, which have been noted in six separate stocks, are striking in that each culture seems to behave as a unit. All these changes were in the one direction; type I changing to type II. In stock 28, where the two complementary mating types had been isolated from a selfing clone, the type I sub-clone eventually reverted to type II at 25°C. While a sub-culture at 18°C remained type I even when transferred to 25°C. There were no signs of any massive death and consequent replacement of one type by another in these cultures. It would seem that subject to microenvironmental conditions, the type I cultures revert to type II which can perhaps be regarded the more stable state. Gilman's (1939) data of the different frequency of change of the two mating types agree with this.

Later one reactive F1 caryonide from a high viability cross (X31 and 28) behaved in a

manner which may be relevant to the mass change of mating type described above. This clone when first extracted reacted as type I but at a later period was found to react as type II. An epidemic of selfing appeared in this culture and pairs were isolated, but all the exconjugants died. Conjugating pairs were then split, isolated, and tested for mating type, with the results shown in the following diagrams:-



On starvation selfing occurred again in these separated lines. Pairs were again split and the following results obtained:-



There were no signs of any nuclear breakdown (indicating autogamy) preceding the selfing process. Further, by outcrossing to reactive type I and type II stocks, conjugation and mating reaction was only shown with the type II stocks. Some of the abnormal type I clones which had passed through the selfing

phase reacted as type II clones, others remained type I or became non-reactive.

One explanation of the behaviour of this clone is that it possesses a genotype such that subject to certain microenvironmental conditions the mating type becomes unstable and conjugation can occur between animals of the one line. Subsequently according to the environmental fluctuations mating type I is either stabilized or reverts to mating type II. In this way the above data may provide a model for the mass shifts of mating type described previously; i.e. some unstable phase can occur when a switch from one mating type to the complementary type is possible, the one eventually stabilized depending on the microenvironmental conditions existing at that time in the culture tube.

(v) THE ABSENCE OF AUTOGAMY.

A nuclear reorganization process in P. caudatum occurring in the absence of conjugation was described by Woodruff and Erdmann (1914), and named by them 'Endomixis'. This nuclear process always coincided with the low points of a long term rhythm in the division rate. Woodruff and Erdmann found it impossible to culture the organisms in depression slides indefinitely. The endomictic stages, which may be more sensitive than the other

stages, could only be discovered by culturing the animals in small tubes during this period. The stages described as being part of the endomictic process had previously been regarded by Hertwig and others as depression stages. Chejfec (1930) reported a rhythmic nuclear reorganization process from observations on mass cultures. The period between peak percentages of animals with fragmented macronuclei was 25-35 days. Fermor-Adrianowa (1925) observed a nuclear reorganization process in cultures after three months mass culture. The only report of such a process using a daily isolation process is that of Galadjief (1932). Galadjief found that an irregularly appearing nuclear reorganization process occurred in some of his lines, but there was no coincidence with the low points of the division rhythm. Some of Galadjief's cultures were continued by daily isolation for more than a year without the appearance of any reorganization process.

The evidence for the existence of a nuclear reorganization process in P. caudatum comparable to the autogamy of P. aurelia is not conclusive since experimentally the possibilities of selfing and degeneration have not been rigidly excluded. More recent investigations by Diller (1940) and Gilman (1941) on American, Orlova (1941

and 1947) on Russian, and Chen (1944) on Chinese stocks of P. caudatum, all following the daily isolation procedure, have not been able to detect any process in the normal vegetative life cycle of this species.

In the present instance, taking an exconjugant clone from a high viability cross (1A of stocks and X22) as a starting point, a serial isolation line was begun with the aim of demonstrating the existence of any nuclear reorganization process in the European collection. The culture was carried in daily isolation for 179 days, representing about 430 divisions, without the appearance of any reorganization process. On three occasions, at the 61st., the 88th., and the 162nd day a few animals with fragmented macronuclei were observed in the starved slides - 3%, 4%, and 2% respectively. At the 61st day sixty animals from the depression with the 3% macronuclear breakdown were isolated, fifty-two divided and the first division products were stained showing no macronuclear breakdown, the other eight when stained on failing to divide after 72 hours all showed macronuclear breakdown. These latter may have been degenerating animals. At any rate there is definitely no evidence for the existence of a regular reorganization process. Two other stocks were similarly observed over periods of three months

with negative results.

Whether autogamy or a similar process can occur in P. caudatum cannot be excluded on the basis of negative data, but it is sure that there is no cyclical process, under conditions of laboratory culture, comparable to that regularly found in all stocks of P. aurelia.

(vi) THE ANTIGENIC PROPERTIES OF P. CAUDATUM.

Antisera against several different stocks of P. caudatum were prepared and tested. Like P. aurelia each individual stock of P. caudatum is able to manifest a number of different, serologically distinct antigenic types. For example, from stock 24 antisera for three different antigenic types were prepared and the existence of another antigenic type (negative to the prepared sera) was inferred. Other stocks showed a similar diversity of antigenic type. There is no evidence of a rigidly controlled temperature series like that found in variety 1 of P. aurelia. In stocks 21, 24, and 43 of P. caudatum, two different types of each stock could be cultured at both 18°C and 25°C for long periods.

Because of the technical difficulties involved in the mating of stocks of P. caudatum, it has not been possible to study the genetic basis of

TABLE II. (a) ANTIGENIC SURVEY OF STOCKS OF P. CAUDATUM AT 25°C.

Sera (1/50) \ Stocks	24			43		21																														
	24a	24b	24c	43a	43b	21a	21b	35	22	3	28	39	1A	5	46	18	32	60	J2	J7	7	10	15	20	31	33	42	47	61	J1	J8	J3				
Anti-24a	10	25		60									30	35																						
Anti-24b	30	8											3	35	3				0/15																	
Anti-24c		40	2							20										25																
Anti-43a				8																																
Anti-43b			8		6										25				0/5	25																
Anti-21a						6		6	15	20	8	15				0/20	40	0/10																		
Anti-21b							1					40																								
Anti-35a						8		8	8	8	15	6								10/40																
Anti-90G (P.aur. V1)						3		8		3	6	10								5																

Figures represent time of immobilization in minutes.

(b) THE STOCKS GROUPED ACCORDING TO THEIR REACTION WITH THE PREPARED SERA.

?	?	?	'G'	?	?
24a	24b	24c	21a	21b	43a
	1A	(43b)	35		
	46		22		
			3		
			28		
			39		

the antigenic diversity or to test for any allelomorphic relations between the types in different stocks.

However by surveying the serological reactions it is possible to group certain types together. The cross-reactions are shown in Table IIa where all the stocks of P. caudatum have been tested against the prepared antisera. A number of different antisera obtained from different stocks of P. aurelia variety 1 were also tested, but only one (the anti-90G) showed any reaction against P.caudatum.

The groups that can be deduced from the table of cross-reactions are shown in Table IIb; one group - the 'G' group - is distinct, the others less so. The 'G' group comprises six stocks which are immobilized by the anti-21a (P. caudatum), the anti-35a (P. caudatum), and the anti-90G, (P.aurelia) sera. (The anti-21a and anti-35a sera of P.caudatum in their turn can immobilize stock 90 type G of P. aurelia at low titre). In the absence of any direct genetical analysis, it would seem probable (comparing the situation here to the system analysed in P. aurelia) that the types bracketted in the 'G' group bear an allelomorphic relationship to one another. The non-G antigenic types of these stocks are therefore presumably determined by other genes which are expressed under different cytoplasmic conditions.

The animals were subjected to non-lethal degrees of immobilization with their homologous antiserum and transformation of type subsequently occurred. This method has previously been used to obtain transformations of variety 4 of P. aurelia (Sonneborn 1947b, and Sonneborn and LeSuer 1948). In this way type 24c of P. caudatum transformed after a few divisions to type 24a, which in its turn could be transformed to 24b; 24b when treated yielded a negative type. Although types 24a and 24b can be maintained for an indefinite period in a temperature range from 18°C to 28°C, the 24c → 24a → 24b transformation series seems constant.

From this it seems likely that the system of control of antigenic traits in P. caudatum is not very different from the situation prevailing in P. aurelia. There is even a remarkable similarity in the serological specificity of the 'G' types of P. caudatum and the G_4 type of stock 90 of P. aurelia variety 1.

5b. DISCUSSION OF WORK WITH P. CAUDATUM.

The first question to be considered is whether the species P. caudatum is differentiated into varieties and how such varieties can be defined. As pointed out earlier the variety unit found in P. aurelia ranks as a physiological sub-species. Mating reactions occur only sporadically between varieties and in these cases either the F1 or the F2 generation is virtually completely inviable. Thus each variety is sexually isolated from the others. Slight cross-reactions are sufficient to indicate the homologies of the two alternative mating types in most of the nine varieties (Sonneborn 1947a).

It is a curious fact that all the stocks of P. caudatum collected should be of the same variety, particularly since there are reputed to be thirteen (or, according to the unpublished work of Gilman, more than sixteen). The varieties of P. aurelia are widely distributed about the world and it is common to find more than one of them in the same body of water. In fact varieties 2 and 9 have been collected from Blackford Pond - the pond which yielded the successfully interbreeding stocks of P. caudatum.

If the varietal concept is to be applied to P. caudatum it would have to be supposed that the crosses of stocks giving exconjugants of high or complete viability represent intravarietal matings, while those of nil or low viability are inter-varietal matings.

But the data from the European stocks of P. caudatum do not necessarily suggest the existence of any discrete units. In table I it is shown that stocks taken from one body of water gave, when mated, exconjugants of uniform high viability as opposed to varying amounts of viability on outcrossing. This has the likeness of a situation which can be represented as an incompatibility system, where the success of conjugation varies with the genotypic similarity of the conjugants.

Whether this idea of mating compatibility depending on genotypic similarity can be applied to the species as a whole or only to the variety 2 complex of Gilman's system is not known. It may be that further study would reveal mating cross-reactions between all the varieties of the species. At any rate it seems better to regard variety 2 as a continuously varying system rather than as a discontinuous one. In order to sustain the latter alternative Gilman found it necessary to propose that each of the European stocks was a variety in its self. At such a point one begins to question the whole concept of 'varieties' in P. caudatum. Further, the nature of the mating reactions within this complex requires reinvestigation. In the European material as described earlier there is variation in the achievement and the outcome of conjugation between

any two stocks at different times. It is comparable variation which is used by Gilman to subdivide the variety 2 complex. (One of the cross-reactions reported by Gilman reacts in an anomalous manner with respect to the rest and to the intervarietal cross-reactions known in P. aurelia. In this case a mating type (XX) of one sub-variety reacts with both mating types (XVII and XVIII) of another sub-variety. The validity of this observation has been questioned by Metz (1954). As the mating type is not a consistent enough character for the subdivision of the species an attempt has been made to use the viability of the exconjugants for this purpose. However the evidence, though rather meagre, tends to favour the continuously varying type of system.

The cytological analysis of the situation, which shows that meiosis is apparently normal and the post-fertilization divisions abnormal, suggests that the mechanism of incompatibility depends on some kind of nucleo-cytoplasmic interaction; particularly since it is the F1 animals which are inviable. It is possible that under conditions of ecological isolation the nature of the cytoplasm as well as the nuclear material can alter in such a way that, as a result of crossing, the heterozygous nuclei are unable to develop normally in a foreign cytoplasm.

On the other hand the fact that certain

stocks (e.g. 24 and 28) were also inviable after selfing is more easily explained on a hypothesis of nuclear abnormalities, where for instance dominant lethal genes may be carried latent in the micronucleus to be expressed only at the time of formation of the new macronuclear elements after conjugation. Certain stocks of the ciliate Tetrahymena pyriformis likewise produce inviable progeny after selfing, but other stocks have been found which give viable offspring after selfing (Elliot and Hayes 1953). The selfing process in P. caudatum has not been examined in detail owing to the sporadic nature of its occurrence. The cytology has not been studied and it is not known whether the same aberrant macronuclear behaviour is present. Most selfing stocks however gave completely viable offspring. Therefore the total inviability of the selfers of stocks 24 and 28 may be a consequence of some derangement of the selfing process, or may be due to nuclear abnormalities present in each stock (as suggested above).

Autogamy has not been found and there has been no sign of ageing. In P. aurelia ageing is presumed to result from the unbalancing of the genotype of the cell macronucleus by its repeated amitotic fissions (Faure-Fremiet 1953, and Sonneborn 1955). Autogamy by reconstitution of the macronucleus counteracts the ageing process. Since

autogamy or ageing is not found in P. caudatum it may be that this species possesses a more efficient mechanism of macronuclear division.

The question also arises whether sexual reproduction is of importance to P. caudatum in the natural environment. In the laboratory sexual behaviour has been very difficult to induce as compared with P. aurelia, and autogamy - omnipresent in the latter - has not been detected. If the macronucleus is presumed to be a highly polyploid structure, organisms of this type will be cushioned from the effects of the accumulation of deleterious mutations. In addition since paramecia have some powers of non-genetic adaptation the sexual reassortment procedure may have fallen into disuse under the influence of a moderately stable environment. However such a possibility seems very unlikely as conjugation does occur in certain instances and viable offspring are produced.

A reservation should be added that the difficulties involved in the breeding of P. caudatum in the laboratory and the abnormalities obtained may be a consequence of some deficiency or unbalance of the culture medium.

The mating type system of P. caudatum seems similar to that of P. aurelia. However the change

of mating type during the vegetative phase has not been found in the P. aurelia material and probably indicates that the mating types in P. caudatum are more completely under cytoplasmic control. The meagre information on the mating types of exconjugant clones tends to support this view. Failure to note this kind of mating type change may have led to the confusion over the variety system and the anomalous cross-reaction in the variety 2 complex noted above.

The antigen system of P. caudatum, without however any genetical proof, appears to agree with the situation in P. aurelia. It is interesting to note that there are serological similarities between the two species, i.e. the two 'G' type sera of P. caudatum, anti-21a and anti-35a, are effective against the G type of stock 90 of P. aurelia, and the serum against the latter in turn immobilizes P. caudatum. Anti-90G was the only serum derived from P. aurelia which was found to cross-react with the stocks of P. caudatum. Animals of type 90G experimentally are good antigens, giving serum of high antibody concentration. The antiserum has a low specificity in that it will react with the 'G' form of P. caudatum (and also, as will be described on pages 83-85 the G type of variety 9 of P. aurelia). It is specific however in the sense

that it shows little cross-reaction with the other G, types of variety 1 of P. aurelia. Because of this, it cannot be decided that the antigenic types in other stocks of P. caudatum not reacting with the anti-90G, anti-21a, and anti-35a sera are determined by different gene loci. The only indication of the other gene loci is from the formation of different antigenic types in the same stock. The genetic homologies of the different types cannot be deduced except in the cases where positive cross-reactions have been detected. In these cases it seems reasonable to assume the serologically similar types are determined by genes at the same locus as has been proved experimentally in P. aurelia. However, genetic homologies can only be established with certainty by breeding experiments.

It will be noted that the G antigenic types in the two species are serologically more similar than certain of the different types found in each individual species. This is a general principle in serology. Many antigenic substances have a wide distribution, as for instance the blood types of humans which can be detected in various species of Primates (Boyd 1943).

6a. EXPERIMENTAL WORK WITH P. AURELIA VARIETY 9.

(i) INTRODUCTION.

A ninth variety of P. aurelia has been described by Beale and Scheller (1954). The original seven stocks from which the variety was described were collected in Scotland. Later stocks collected in France near Paris were also found to belong to variety 9. This variety has not been recorded in the extensive collections made on the American continent or as yet from any other part of the world. P. aurelia was earlier considered to be rare in Scotland, but variety 9 has been found to be comparatively abundant at certain times of the year and it has been possible to make a preliminary ecological study of a population in an enclosed body of water.

Work on the antigenic characters of P. aurelia variety 9 has been carried out along the following lines:- A rapid survey of the antigenic variation has been made comparing the situation in this variety to that in the varieties previously studied. The genetic basis of the variation has been analysed and the similarities to and the differences from the situation in varieties 1 and 4 noted. Variety 9 resembles variety 1 more than variety 4, (for example; mating type system, inter-autogamous interval, etc.), and since stocks and

antisera of the variety 1 material were available in the laboratory, a comparison of both varieties could be made to see to what extent they are comparable serologically. Variety 1 and variety 9 are reproductively isolated and rank as sub-species, but it can be presumed that evolutionarily they are not long divergent and an attempt has been made using serological techniques to establish the homologies of genes and alleles in the two varieties.

Paramecia exhibit a great deal of antigenic variation. The antigenic polymorphism is determined genetically by several loci with several alleles at each locus. Since many multiple allele systems in other organisms have now been shown to be systems of 'pseudoallelic' factors of closely linked genes, an attempt has been made to demonstrate this with respect to the antigen type determinants of P.aurelia. The detection of pseudoallelism would indicate a path by which the polymorphism of each gene has arisen and the relations of the existing allelic factors of a gene.

(ii) GENETIC BASIS OF THE ANTIGENIC VARIATION OF VARIETY 9.

Variation within Stocks.

The work of Sonneborn (1950a) with variety 4 and Beale (1952) with variety 1, referred to previously, has shown that each stock of P.aurelia can display more than one antigenic type. Since a

stock is derived from a homozygous individual, this intra-stock variation is not the result of the substitution of nuclear genes. It has been shown that it is due to cytoplasmic factors.

The system of variation of antigenic type in variety 9, as will be shown below, is similar to that found in both varieties 1 and 4. If a stock of variety 9 (e.g. 509) is injected into a rabbit an antiserum (anti-509G) is obtained which will immobilise animals of the type injected - designated type 509G.** Some animals will be found which are not immobilised by this antiserum, and a second antiserum -- anti-509HT - can be produced by injecting these resistant animals. This antiserum will immobilise animals of the type injected (509HT) but not animals of the first type (509G). Similarly other antigenic types can be found in the one stock. However, in stock 509 of variety 9, the antigenic types 509G and 509HT are the ones most usually found. Likewise in all the other stocks of variety 9 examined two antigenic types - a G type and an HT type - predominate.

Influence of the Environmental Conditions.

When animals of stock 509 are grown in depression slides at temperatures between 14°C and 28°C the G antigenic type is usually found. If

** Reasons for this nomenclature (e.g.509G,509HT,etc) will be explained on page 78.

the animals are grown at temperatures between 31°C and 33°C (33°C is the upper limit of survival of variety 9) the HT antigenic type is usually found. A transformation of one type to another can regularly be produced by putting the animals at the appropriate temperature. When animals of type 509G are transferred from 25°C to 31°C the transformation to type 509HT occurs after approximately ten fissions. The reverse transfer of HT type animals from 31°C to 25°C brings about the transformation to G type after about seven fissions. By transferring 509HT animals from 31°C to lower temperatures the transformation to type 509G results after fewer fissions but in a longer time (Table III).

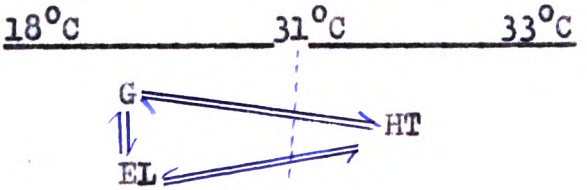
TABLE III. TRANSFORMATION OF THE HT TYPE OF P. AURELIA V.9., TO THE G TYPE.

Temperature shifts from 31°C to:-	Completed transformation from HT to G type.	
	Approx. No. of divisions.	Approx. length of time in days.
25°C	7	2.5
18°C	4	3
14°C	2-3	7
5°C	1-2	12

It was not possible to induce transformation without division. (In this respect the antigen transformations of Paramecium are different

from those of *Trypanosoma*, described on page 22 which occur easily without division.) The G type antigen can be detected in some animals before the first division, but the transformation is never complete till some time after division. This HT→G transformation system of variety 9 resembles the D→B system studied by Beale (1948) in variety 4. The HT→G transformation is a temperature dependent reaction, and it is also 'division dependent' in the sense that it requires the structurally modifying effects of at least one division before transformation of type can be achieved.

In stock 509 other antigenic types occasionally arise. For instance the G type of stock 509 sometimes changes to an EL type at 25°C. This EL type when put at 31°C transforms to the HT type. The reverse transformation of this HT type will produce either type G or type EL. Thus a triangular system can be drawn:-



The EL type however is unpredictable in its appearance and some other factor than the correct temperature is necessary for its manifestation.

A factor found to affect the stability of the antigenic type is the oxygen content of the

culture medium. Animals which are G type in depression slides rapidly change to another type when grown in flask cultures at the same temperature. It is possible to prevent this transformation by growing the larger volumes of culture required for injection in shallow vessels (thereby increasing the surface area) or by passing a stream of air through the cultures. The HT antigenic type reacted similarly and it was necessary to grow the material for injection in aerated cultures.

Transformation of one type to another did not usually occur in P. aurelia variety 9 after immobilization with homologous antiserum, although this technique has been used successfully with other material e.g. certain types of variety 4 (Sonneborn and LeSuer 1948), and with P. caudatum (see page 58).

The environmental conditions necessary to maintain any one antigenic type are complex and the number of antigenic types in a stock is obviously more than the two found with the culture conditions used in this work.

Variation between Stocks.

All the stocksof variety 9 which have been collected behave similarly in their response to the variation of temperature. All characteristically produce two antigens: a G type which

transforms to an HT type between temperatures of 28°C and 31°C. Some of the 'corresponding' antigenic types of the various stocks show a serological relationship and some are serologically distinct. The reason for using the G and HT nomenclature is based on the genetic relationships and will be explained below.

Seven antisera have been prepared against the G types of seven stocks of variety 9. In Table IVa the homologous reactions are shown along with the cross-reactions found ~~between them~~. (The survey was carried out at the one time to avoid variations due to different serum lots). Stock 503 and stock 504 behave identically and the sera prepared against them react in the same way; likewise stock 510 and stock 518 are similar. Thus the G types can be grouped serologically into five sub-groups (Table IV b). Each sub group of the G types is serologically distinct, except groups b and c which although similar can be distinguished by their cross-reactions with non-homologous sera. The remainder of the stocks of variety 9, to which no antisera were prepared, can all be placed in one or other of these five sub-groups. No other G antigenic types of different serological characteristics have been found in the stocks of variety 9 at present available.

TABLE IV.

(a) REACTIONS OF THE G ANTIGENIC TYPES WITH ANTI-G SERA (P. AURELIA VARIETY 9).

Antisera \ Stocks	503	504	505	506	509	510	518
Anti-503(1/50)	4	4	s	100	s	s	s
Anti-504(1/50)	4	4	120	50	s	s	
Anti-505(1/50)	5	10	3	3	15	4	3
Anti-506(1/50)	vs	30	8	3	vs	100	s
Anti-509(1/50)	50	50	vs	60	3	90	vs
Anti-510(1/50)	vs	s	s	60	s	5	2
Anti-518(1/5)	s	vs	vs	vs		3	3

(Figures represent time of imm. in minutes. s = slow movement, vs = very slow movement).

(b) GROUPING OF G TYPES BY SIMILARITY OF REACTION.

Group a	Group b	Group c	Group d	Group e
503 504	505	506	510 518	509

Eight antisera have been prepared against the HT types of the eight stocks of variety 9. In Table Va the homologous reactions of these eight stocks and antisera are shown along with the cross-reactions found between them. Only stocks 504 and 509 react identically. The remainder are quite distinct. There is also less cross-reaction between the HT types than there is between the different G types. The HT types can thus be grouped into seven sub-groups (Table Vb). All the other stocks of variety 9, for which no antisera were prepared, can be placed in one or other of the seven groups.

No serological cross-reactions have been found between antigens of the G types and those of the HT types (see Tables VIII and IX).

Thus at present five G antigenic types and seven HT antigenic types are known to exist, in the different stocks of variety 9. In variety 1 Beale (1954) has been able to subdivide the corresponding types into groups: three S types, six G types and seven D types have been found.

The Gene Differences between Stocks.

The genetic analysis of the antigenic variation of varieties 1 and 4 of P. aurelia has been described by Beale (1952) and Sonneborn (1950a) respectively.

Crosses have been made between different stocks of variety 9. The animals obtained were

TABLE V.

(a) REACTIONS OF THE HT ANTIGENIC TYPES WITH ANTI-HT SERA (P. AURELIA, VARIETY 9).

Antisera \ Stocks	503	504	505	509	510	530	531	532
Anti-503 (1/50)	7			65	s			
Anti-504 (1/50)	30	4		4	s			
Anti-505 (1/50)	90		3	vs				
Anti-509 (1/50)	20	10		10		s		
Anti-510 (1/50)	s	s		100	5			
Anti-530 (1/50)					75	5		
Anti-531 (1/50)		50		45			12	s
Anti-532 (1/50)		vs		90			90	7

(Figures represent time of imm. in minutes. s = slow movement, vs = very slow movement).

(b) GROUPING OF HT TYPES BY SIMILARITY OF REACTION.

Group a	Group b	Group c	Group d	Group e	Group f	Group g
503	504 509	510	505	530	531	532

made to go through autogamy, and the segregation of the antigenic types was studied. When the antigenic types of the parental stocks were distinguishable a mixture of the two antigenic types could be found in the F1 animals after a delay of about five fissions. For instance the F1 animals of a cross of stock 509 and stock 510 formed antigens of both types 509G and 510 G at 25°C, and types 509HT and 510 HT at 31°C. Mixtures of the antigenic types characteristic of the different temperature ranges were not found, except briefly during transformation. For example in the cross of stock 509 and 510, the antigens characteristic of the F1 were either 509G + 510G or 509HT + 510 HT, but not 509G + 510HT or 509HT + 510G.

The exautogamous F2 clones showed a segregation of the antigens characteristic of the parent stocks, e.g. in the cross of stock 509 and 510 only two antigenic types - 509G and 510G - appeared in the F2 at 25°C; at 31°C only types 509HT and 510HT appeared. All the stocks carrying distinctive antigenic types in either of the temperature ranges have been crossed and the segregation of antigenic type is shown in Table VI. The ratios of each of these antigenic types approximates to the ratio of 1:1 expected by the segregation of Mendelian factors. By scoring the antigenic types of the progeny from one cross (509 x 510) in both temperature ranges four classes are obtained(Table VII).

TABLE VI.

SEGREGATION OF ANTIGENIC TYPE IN EXAUTOGAMOUS F2 LINES.

Cross	Segregation of Antigenic Type	
	At 25°C	At 31°C
509 x 510	107/509G 93/510G 20/dead	58/509HT 78/510HT 35/dead
503 x 510	69/503G 64/510G 12/dead	31/503HT 44/510HT 6/dead
504 x 510	83/504G 86/510G 31/dead	51/504HT 52/510HT 17/dead
505 x 510	53/505G 62/510G 5/dead	51/505HT 42/510HT 7/dead
506 x 510	74/506G 50/510G 16/dead	55/506HT 51/510HT 14/dead
530 x 510		70/530HT 77/510HT 3/dead
531 x 510		60/531HT 79/510HT 11/dead
532 x 510		88/532HT 70/510HT 32/dead

TABLE VII.

RECOMBINATION BETWEEN TWO PAIRS OF ANTIGENIC TYPES.

Cross		Parental Types.		Recombinant Types.		Dead.
509 x 510	Type expressed at 25°C	509G	510G	509G	510G	
	Type expressed at 31°C	509HT	510HT	510HT	509HT	
		33	48	30	23	15

Probability of deviation from 1:1:1:1 ratio is >0.01 <0.02 (χ^2 10.16 f = 3).

The classes appear in a proportion not significantly different from the ratio of 1:1:1:1.

The results of these crossing experiments can be interpreted as representing a system of several alleles at two unlinked loci - g and ht. Only one of these gene loci is expressed at any one time; genes of the g locus between 14°C and 28°C, and genes of the ht locus between 31°C and 33°C. There is no sign of any dominance among any of the alleles of one gene. Five alleles of the g gene and seven alleles of the ht gene can be recognised by the serological methods used. Thus the antigenic types which are expressed at 25°C, although they may be serologically different, are designated G types as each is controlled by an allelomorph of the g gene. The serologically distinct types expressed at 31°C are similarly designated HT types as each is controlled by an allelomorph of the ht gene.

The action of varying temperature on the expression of the genes at the g and ht loci - as the following experiment shows - is by modification of the cytoplasm. If animals of type G and type HT are kept at temperatures between 28°C and 31°C, both types may remain stable for long periods enabling crosses to be made. The F1 from the cross of G type animals of stock 509 and HT type animals of

stock 510 contains two classes of antigenic types - 509G + 510G and 509HT + 510HT. The 509G + 510G class are animals which have received their cytoplasm from the 509G parent and the 509HT + 510HT animals have received their cytoplasm from the 510HT parent. These F1 clones are genotypically identical and the environmental conditions are the same. The only difference between them is in the nature of the cytoplasm.

Thus in variety 9, as has already been shown in varieties 1 and 4, the particular gene-loci which is expressed depends on the state of the cytoplasm, and the expression of each member of one allelomorphic series is favoured by the same cytoplasmic state.

Test for Recombination between Alleles.

The presence of two multiple allele systems in P. aurelia variety 9 has been demonstrated. In other organisms some multiple allele systems have been discovered to be groups of tightly linked genetic factors (e.g. the pseudoallelic series of the vermillion locus in Drosophila (Green 1954). Crossing-over only occurs rarely between these genes and therefore on first analysis the genes may be assumed to be true alleles. If, however, the products of meiosis are examined on a large enough scale low frequency recombinant types can be found.

The allelomorphs of these closely linked genes may show a position effect. If the two genes are one on each of the two homologous chromosomes of a diploid they act as homozygous recessive allelomorphs. If as the result of a crossing-over the two genes are located on the one chromosome, they now act as two separate recessive heterozygous genes. Hence these factors are described as 'pseudoalleles'.

To test the possibility of the occurrence of crossing-over between the genes determining the antigenic characters of P. aurelia, a large scale recombination experiment was devised. Stock 509 and 510 whose G types are serologically distinct were chosen for the attempt to find recombinant types resulting from the crossing-over of pseudoallelic genes. If the factors determining these antigenic types are pseudoalleles, a cross-over between them might be expected to produce phenotypically two new antigenic types, one probably reacting against the parental type antisera (anti-509G and 510G), and the other against neither sera.

Three sera were used to screen the products of meiosis; they react as follows:-

	Parental types.		Expected cross-over types.	
	(Stock 509)	(Stock 510)	(type 1)	(type 2)
anti-509G	+	-	+	-
anti-510G	-	+	+	-
anti-505G	+	+	+	+

The third serum - anti-505G - is included since it reacts against all the known G antigenic types (see Table IVa), and therefore can be used to differentiate the cross-over negative types from the types which react negatively owing to their transformation to a type determined by a different gene.

P. aurelia although a diploid organism is very suitable for this type of experiment as autogamy involves meiotic division and the animals at the same time are made homozygous. In the preparation of the experiment stock 509 was crossed with stock 510 and the F1 obtained was backcrossed to stock 510 in order to isolate the heterozygous G locus in an approximately uniform genotypic background. Animals of the seventh, eighth, and ninth backcross generations were used in the course of the experiment which involved merely passing these lines through autogamy and scoring large numbers of exautogamous clones against the three antisera to screen out cross-over types. 7542 clones were examined and were classed into two types:-

	type a	type b
anti-509G	+	-
anti-510G	-	+
anti-505G	+	+
	3735	3807
	7542	

These types are the parental types, no cross-over

types of the kind considered above were found. The ratio of the two types does not differ significantly from the expected Mendelian segregation. Two clones reacting positively to all three sera were found, but both died out at the next autogamy or before, and possibly carried gross chromosome abnormalities resulting from nuclear maldivision.

The efficiency of this experiment was impaired because of the high proportion of clones which transformed to some cytoplasmic type other than the G type. Experimental lots where the percentage of transformations was high were discarded, in the rest the non-G types were followed up and scored for their G type genotype on transformation. A considerable amount of mortality was encountered mainly due to the ageing of the heterozygous clones. Consequently the heterozygous lines had to be back-crossed at intervals in the course of the experiment to counteract the ageing effect.

The result of this experiment is that if cross-over types of the kind considered exist, they do not arise at a frequency above approximately 1 in 7500. To examine the possibility of their occurrence at lower frequencies would require the selection of more stable antigenic types which will not transform so readily and design of the experiment so that it can be carried out on mass cultures.

(iii) SEROLOGICAL COMPARISON OF THE ANTIGENIC VARIATION OF VARIETY 1 AND VARIETY 9.

In variety 1 there are three common cytoplasmic states; from 10°-18°C the S antigenic types are found, from 18°C - 27°C the G types, and from 25°C - 35°C the D types (Beale 1954). In variety 9, as has been described, there are two usual cytoplasmic states, the G types from 14°C - 28°C, and the HT types from 31°C - 33°C.

A comparison of the two varieties serologically might provide a means of indicating homologies between the determinant genes and alleles in each. (Definite homologies can only be inferred as the result of genetic analysis, but since the varieties are genetically isolated, serological comparison is the only approach possible). To this end the variety 1 stocks were tested with variety 9 antisera, and the variety 9 stocks were tested with variety 1 sera.

I. The G types. First the G types of each variety were compared, since they are stable in the same temperature range and from preliminary work cross-reactions were known to exist. The reactions are shown in Table VIII.

There are no cross-reactions between the HT type antisera of variety 9 and the G type animals of either variety 1 or variety 9. However there is some cross-reaction between the G types of each variety. All the cross-reactions are of a low order compared

TABLE VIII. COMPARISON OF G TYPES AND SERA OF VARIETY 1 WITH VARIETY 9.

Anti-Sera.	G-Type Sera of Variety 9.					G-Type Sera of Variety 1.							HT-Type Sera of V.9			
	Anti-504	Anti-505	Anti-506	Anti-509	Anti-510	Anti-41	Anti-60	Anti-61	Anti-90	Anti-92	Anti-156	Anti-168	Anti-503	Anti-505	Anti-509	Anti-510
Dilution. Stock.	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/50
Var. 9 504	4	10	30	50	s			120	2		90					
505	s	3	8	vs	s			90	2					s		
506	50	3	3	60	60	s		30	2							
509	s	15	vs	3	s				4							
510		4	100	90	5			20	2		vs					
Var. 1. 41		20	vs	120	12	20		35		s						
60							16	120	120		s					
61		12	80	120	10	80		4		s						
90		10	s	s	20			s	2		s					
92										20						
156	120			vs				s			25	vs				
168	vs										s	7				

(Figures = times of immobilization in minutes. s = slow, vs = very slow movement).

with the homologous reactions. The anti-90G serum of variety 1 is exceptional in that it immobilises all the sub-types of variety 9 indiscriminately. The variety 9 antisera are however not reciprocally effective against stock 90 of variety 1. This anti-90G serum also reacts with certain antigenic types of P. caudatum. (see page 57).

It is easily seen that there is a limited relationship between the G types of each variety, enough probably to indicate that a homologous gene is involved. However there is no one to one correspondence of the different sub-types in each variety which would indicate the homologies of allelomorphs.

II. The High Temperature Antigenic Types. In variety 1 the D antigenic types are expressed in the high temperature range and in variety 9 the HT types. Three of the commonest D types of variety 1 with their homologous sera and all seven HT sub-types of variety 9 were used. As before the variety 1 sera were tested against the HT type stocks of variety 9 and reciprocally the variety 9 sera were used against the D type stocks of variety 1. The reactions are shown in Table IX.

The G type sera of variety 9 show virtually no cross-reaction with the high temperature types of either variety 1 or variety 9. Contrary to the previous case there are also no reactions between the D types of variety 1 and the HT types of variety 9.

TABLE IX.

COMPARISON OF HT TYPES AND SERA OF V. 9 WITH D TYPES AND SERA OF V. 1.

(Figures = time of immobilisation).

Antisera	HT type sera of variety 9							D type sera of V.1			G type sera of variety 9.				
	Anti-503	Anti-505	Anti-509	Anti-510	Anti-530	Anti-531	Anti-532	Anti-60D	Anti-90D	Anti-192D	Anti-503	Anti-505	Anti-506	Anti-509	Anti-510
Dilution. Stocks.	1/50	1/50	1/50	1/50	1/50	1/50	1/50	1/100	1/100	1/50	1/50	1/50	1/50	1/50	1/50
Variety 9															
503HT	7	90	20												
505HT		3													
509HT	65		10	100		45	90								
510HT				5	75										
530HT					5										100
531HT						12	90								
532HT							7								
Variety 1															
60D								7							
90D									20						
192D										7					

This may indicate therefore that the two genes concerned in the determination of the high temperature antigenic types are not homologous in the two varieties. However this is not certain in view of the large amount of intra-varietal variation.

The conclusion to be drawn is that both varieties are considerably divergent in their antigenic characteristics. The antigens carried in the middle temperature range show considerable similarity but they are not identical. The high temperature types of each variety are not similar, and if the genes determining them are not homologous the temperature sequence of expression of the types is not the same in each variety.

(iv) GEOGRAPHICAL DISTRIBUTION AND ANTIGENIC VARIATION.

The antigenic variation of variety 9 has been surveyed on pages 71-74 and now the characteristics of its distribution in nature will be considered. Table X is a list of the extant stocks of variety 9, their geographic source, and the G and HT antigenic type of each stock. (Eighty stocks from Blackford Pond will be considered separately on pages 90-97).

It will be seen that in some cases - 505G and 532HT - the particular antigenic type is limited to one locality. In other cases the antigenic type has been recorded from more than one place, e.g. type 509G originally discovered in stock 509 in Scotland subsequently appeared in material collected

TABLE X — DISTRIBUTION OF THE ANTIGENIC TYPES OF VARIETY 9 OF P. AURELIA.

Number of Stocks collected.	Place Collected.	Number of each type	G-antigenic type	HT antigenic type
2	St. Margaret's Loch, Edinburgh	1	503	503
		1	503	504
1	Figgate Pond, Edinburgh		503	504
1	Strathblane, nr. Glasgow		505	505
1	Canal, Kingsknowe, Edinr.		506	504
1	Canal, Ratho Park, Edinburgh.		509	504
1	Chantilly, France		510	505
2	Gif Pond, Gif, France	1	510	510
		1	510	504
1	Cernay-la-Ville France		510	532
9	La Fillolière, nr. Paris	6	509	510
		1	509	503
		1	510	503
		1	510	510
2	Blackford Pond, Edinburgh		510	510
80	Ditto - May '54 - Jan '55	See Table IXa		

in France.

The number of places where variety 9 has been found is limited but the material is sufficient to show that the antigenic types are not restricted geographically. The majority of the types occur in France and Scotland, and it is very probable that those types which are at present unique, will be found in other places as the result of more extensive collections.

There is no correlation between the G antigenic type and the HT antigenic type found in a particular stock. For instance antigenic type 503HT was originally found in stock 503 in association with type 503G; in stocks collected since then it has been found in association with types 509G and 510G.

Similar results for the S, G, and D antigenic types of variety 1 have been published (Beale 1954).

(v) AN ECOLOGICAL STUDY OF THE VARIATION FOUND IN ONE HABITAT.

An intensive study of the variation in one more or less isolated habitat has been carried out. The site was Blackford Pond, a stretch of water approximately 150 by 30 yards. This body of water is isolated from any water course, being fed by spring water from Blackford Hill.

Procedure. The method of sampling was to collect a number of water samples (the average

number was 34) from one section of the pond. The part chosen was a 25 yard strip of the south-west border of the pond which is overhung by foliage and likely to be the most suitable environment for paramecia. After incubation in the laboratory the tubes were examined. If P. aurelia was found in any tube one clone only was isolated from that tube, as the animals of one tube are the fission products of a very few original cells. (In test cases where more than one animal was isolated from the same tube, the animals were always genotypically identical). In order to detect the existence of heterozygotes it was necessary to screen the animals with antisera first and later to verify the variety of the clone. This was necessary to ensure that animals did not pass through autogamy before they were tested. The heterozygotes obtained are animals from the wild population since most pass through autogamy at an earlier period than would be possible if they were the products of matings in the sample tube.

The Variation Found in One Habitat. A total of eighty stocks have been isolated from the pond in the course of one year, and the antigenic types of these stocks are shown in Table XIa. The overall frequencies of the determining alleles are given in Table XIb. (It will be noted that only heterozygotes of the G-type determining gene were detected as

TABLE XI. (a) ANTIGENIC TYPES OF STOCKS FROM BLACKFORD POND MAY '54 - JAN '55.

No. of Stocks of each type	(Antigenic Types) G-Type	HT-type.
1	509	510
12	506	510
2	510	?
47	510	510
10	510	530
1	510	531
5	506/510	510
2	506/510	?

(? = stock died before serotype known).

(b) OVERALL FREQUENCY OF DETERMINANT ALLELES.

Frequency of G type alleles		
g^{510}	g^{506}	g^{509}
63.5	15.5	1
Frequency of HT-type alleles.		
ht^{510}	ht^{530}	ht^{531}
65	10	1

anti-sera against the HT antigenic types were not available when the stocks were collected, and consequently the serotyping had to be carried out some time after the collecting).

Yearly Fluctuation of the Species. The collections have been made during the course of one year from May '54 to May '55 (see Table XII). In that time considerable fluctuations of the paramecium fauna have been observed. Figure 1 is a histogram of the density of two varieties of P. aurelia, of P. bursaria, and of P. caudatum. (The density is measured as the percentage of tubes of the total collection containing a particular species).

P. caudatum predominated throughout the year, while P. bursaria appeared abruptly in August and then rapidly declined. Variety 9 of P. aurelia appeared in high density in September during the period of decline of P. bursaria. A gradual parallel decline of P. aurelia and P. caudatum took place, until at the end of March no paramecia of any species were found. Variety 2 of P. aurelia never appeared in any great numbers.

Collections made at different times during twenty-four hours gave no indication of any diurnal fluctuations in density.

It seems also that the density of the population fluctuates violently from year to year. P. caudatum at high density in May '54 is absent in

TABLE XII. SPECIES NUMBERS IN COLLECTIONS FROM BLACKFORD POND.

Date of Collection.	Collection.	Number of Tubes.	Number of Tubes Containing:- P.aurelia v.2	P.aurelia v.9.	P.bursaria	P.caud
18.5.54	A	31				17(55)
22.5.54	B	25	-			12
	C	25	1 (4)			14(52)
26.5.54	D	20		1 (5)		10
	E	30		-		16(52)
8.6.54	F	30				16(55)
2.7.54	G	25	2 (8)	1 (4)		20(80)
14.7.54	H	25				19(76)
17.8.54	I	24			14(54)	13(54)
1.9.54	J	50	1 (2)	9 (18)	20(40)	40(80)
21.9.54	K	29		12 (42)	4(14)	18(62)
2.10.54	L	30	1 (3.3)	10 (23)		19(63)
16.10.54	M	50	4 (8)	21(42)		30(60)
5.11.54	N	35		8 (23)	2(5.7)	14(40)
24.11.54	O	45		11(25)		14(31)
11.12.54	P	39		5 (13)	2(5.1)	7(18)
5.1.55	Q	50		2 (4)		10(20)
27.1.55	R	50			1 (2)	4 (8)
9.3.55	S	42				1 (2.3
31.3.55	T	55				
20.4.55	U	54				
6.5.55	V	54			4 (8)	
26.5.55	W	50				
15.6.55	X	50				5(10)
7.7.55	Y	50			1(2)	15(30)

Figures in brackets are approx. percentages.

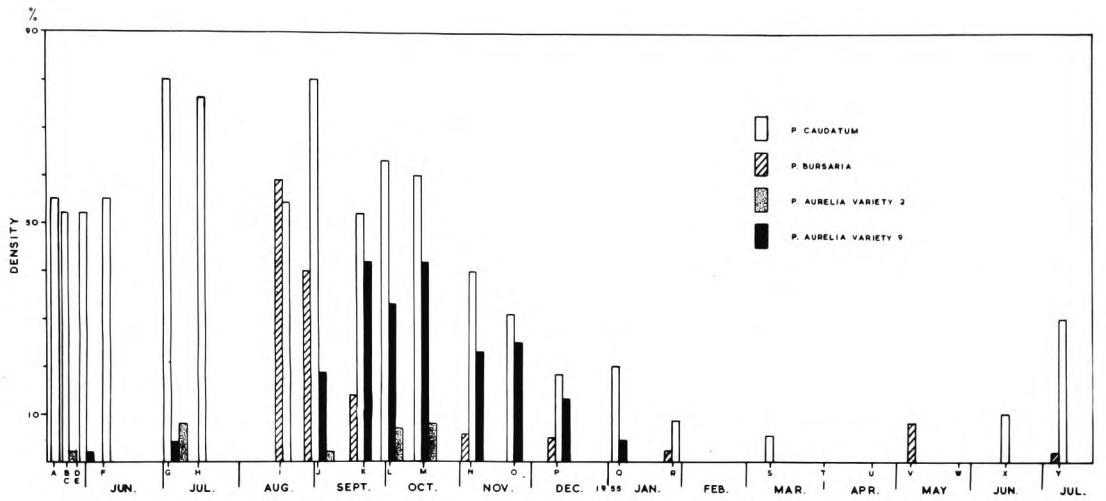


Figure 1. Histogram of the density of each species of Paramecium in the consecutive samples.

May '55 and first appears again in June. The difference is probably the result of the severity of the winter, since the pond was completely frozen over during the whole of February and again for a time in March. A specimen of P. caudatum was collected after five weeks under the ice, so the low temperature itself does not completely exterminate the animals. A similar difference in insect emergence between the two years was observed, presumably also due to the severe winter: very few cast skins were found in May '55, although during the corresponding period in the previous year the surface of the water was thick with a slime of casts.

Genetic variation of the population during one year.

The stocks of variety 9 collected from Blackford Pond have been tested serologically and the antigenic composition of each sample is given in Table XIII.

In Figures 2 and 3 histograms of the density of the different antigenic types through one year are shown. Figures 4 and 5 show the percentage composition of the antigenic types in the population.

Figure 2 shows the frequency of the two homozygous antigenic types and their heterozygote type. The 510G type predominated throughout the year, while the 506G type was present at a lower frequency. Heterozygotes appeared in numbers after the increase

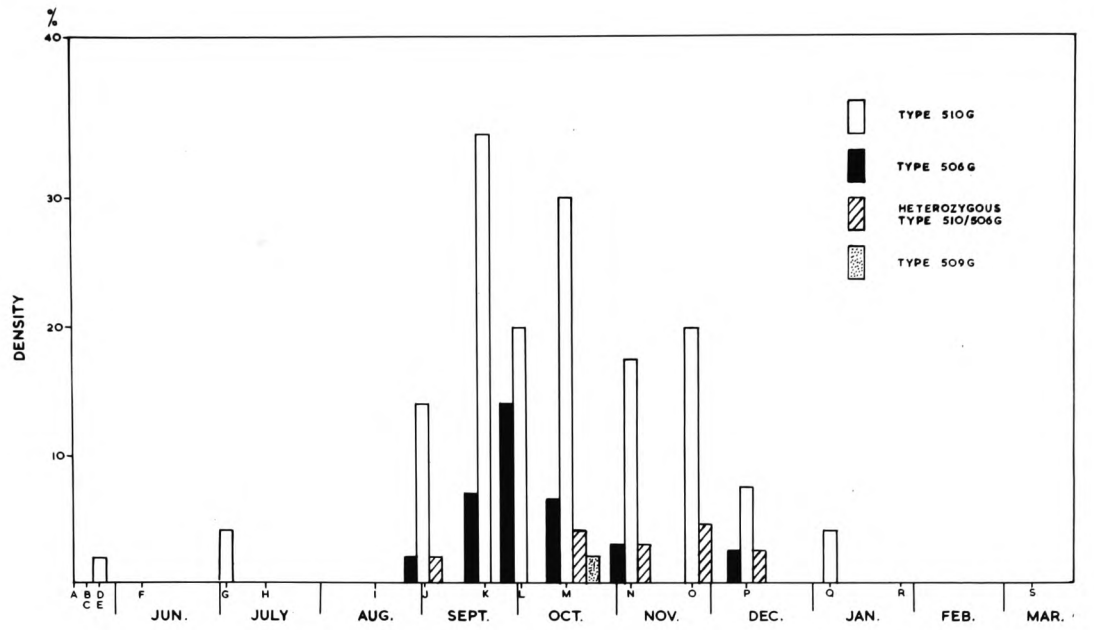


Figure 2. Histogram of the G antigenic types of P. aurelia, variety 9

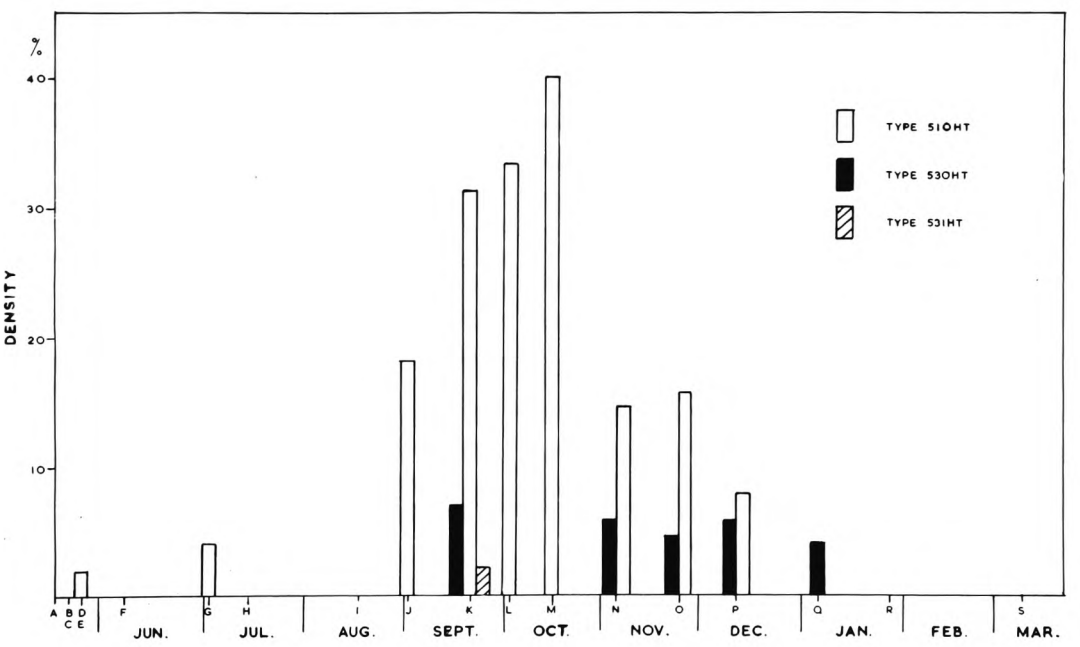


Figure 3. Histogram of the HT antigenic types of P. aurelia, variety 9.

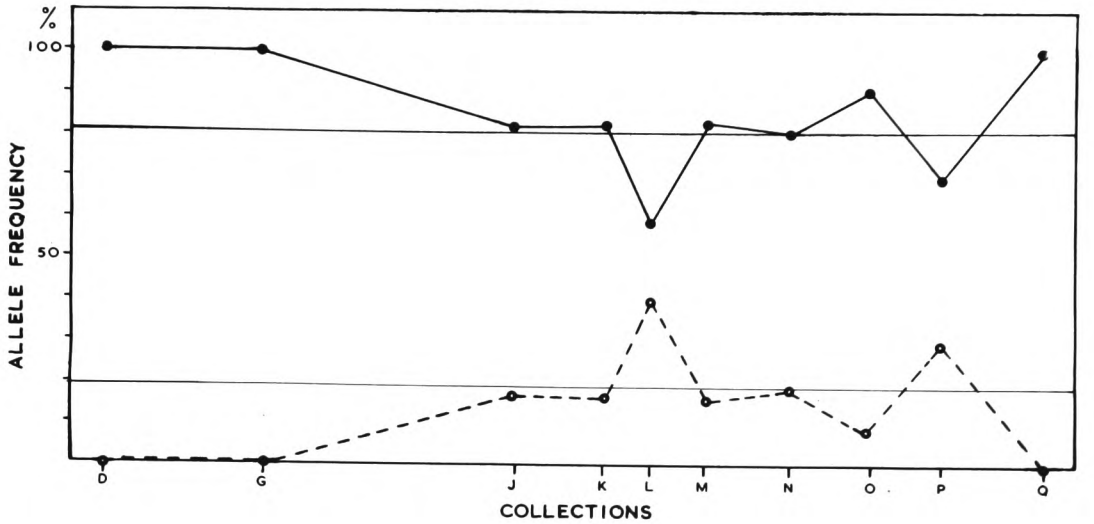


Figure 4. Graph of the proportions of the G antigenic types in the consecutive samples (measured as the allele frequency).

●—frequency of g^{510} o—frequency of g^{506}

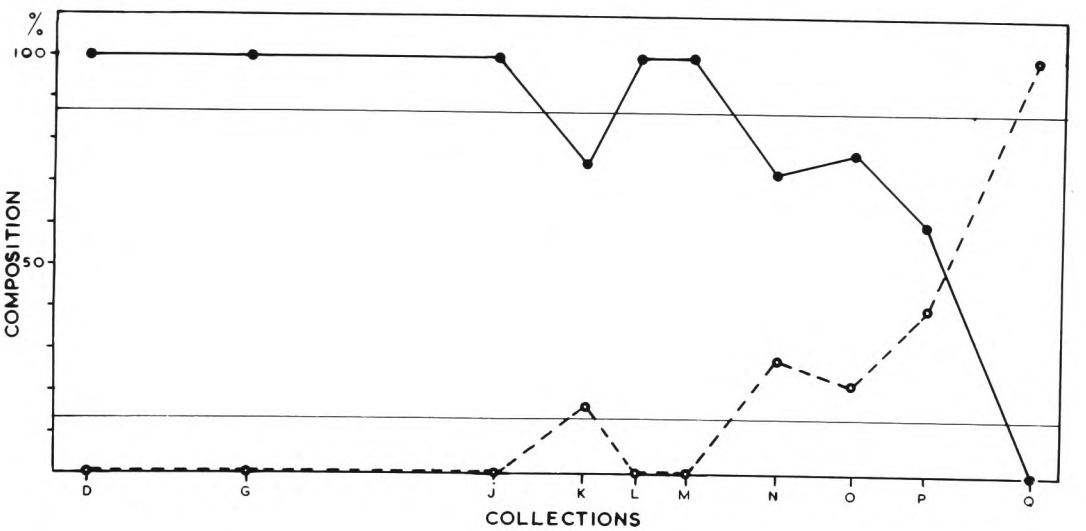


Figure 5. Graph of the proportions of the HT antigenic types in the consecutive samples.

●—type 510HT o—type 530HT.

(Thin lines represent the proportions in the total collection).

TABLE XIII. ANTIGENIC TYPE COMPOSITION OF THE COLLECTIONS FROM BLACKFORD POND.

Collection.	No. of Tubes.	No. with P.aur. v.9.	G Types: No. of stocks of antigenic type. 506G	510G	506G/510G	509G	HT Types: No. of stocks of antigenic type 510HT	530HT	531HT	Dead before tested	Combined G & HT Antigenic Type: No. of stocks of antigenic type. 506G 510HT	506G/510G 510HT	510G 530HT	510G 531HT	510G 510HT	509G 510HT
D	20	1		1			1								1	
G	25	1		1			1								1	
J	50	9	1	7	1		9				1	1			7	
K	29	12	2	10			9	2	1		2		2	1	7	
L	30	10	4	6			10				4				6	
M	50	21	3	15	2	1	20			1	3	1			15	1
N	35	8	1	6	1		5	2		1	1	1	2		3	
O	45	11		9	2		7	2		2		1	2		6	
P	39	5	1	3	1		3	2			1	1	2		1	
Q	50	2		2				2					2			
TOTALS		80	12	60	7	1	65	10	1	4	12	5	10	1	47	1

in density of the population. Figure 4 shows the proportions of the types in the samples (given in this case as the allele frequencies) and no obvious trend is visible. The sample proportions oscillate around the proportions calculated from the whole year's results, and statistically there is no significant deviation (heterogeneity χ^2 = 6.572 for 8 degrees of freedom).

Figure 3 shows the frequencies of the HT antigenic types, and Figure 5 shows the proportions of the types. It can be seen that the 510HT type was replaced in time by the 530HT type. The sample proportions show a trend and differ considerably from the proportions calculated from the whole year's results. Statistically there is significant evidence of a trend (χ^2 of linear trend = 9.978 for 1 degree of freedom).

Each gene has been considered separately and the pattern of variation of each is different. The numbers involved in the experiment are, however, not large enough to be used to demonstrate any trend in the combined G and HT genotype of the population.

No animals of the new summer (1955) population have been found yet, and consequently nothing is known about the antigenic types and their frequencies compared to those of the preceding population.

However two stocks (510 and 524) are available which were isolated in September '52 and January '53 respectively and therefore they may be animals selected from the first and last parts of the population cycle of that year. These animals are of the same genotype, both being of antigenic type 510G, 510HT. The alleles determining the 510G and the 510HT types are the ones with the highest frequency in the '54 - '55 population. Therefore the genetic composition of the populations of '52 - '53 and '54 - '55 may have been very similar. How the increase in frequency of the 530HT type in '54 - '55 will be reflected in the next population remains to be seen.

It should be noted incidentally that the discovery of heterozygotes in a wild population is proof that the laboratory defined unit - variety 9 - is also a functional breeding unit in nature and that crossing occurs in nature.

6b. DISCUSSION OF WORK WITH P. AURELIA VARIETY 9.

The genetic determination of the antigenic characters of variety 9 has been found to be similar to the system already discovered in variety 1 and variety 4. The general situation being that the variation is determined by multiple alleles at several unlinked loci, the particular one expressed depending on one of a number of alternative states. However the details of the situation in variety 9 do not correspond exactly to either the variety 1 or the variety 4 system.

In variety 1 there is a regular effect of temperature on the system of cytoplasmic states. The exact boundaries of the temperature ranges are slightly different in each stock and it is found that the differences are controlled by the alleles which also determine the specificities of the antigens (Beale 1952). In variety 4 the sequence of cytoplasmic states is not so regular and it may be different in different stocks. But the temperature stability characteristics of each stock type is determined by the allele controlling its specificity (Sonneborn,T.M., Ogasawara,F., and Balbinder, E. (1953),). In variety 9 the G and HT types are expressed in a temperature sequence. But variety 9 and variety 1 are distinct because the temperature stabilities of each stock type of variety 9 do not differ; all the G types can be transformed to any

of the HT types at a temperature somewhere between 28°C and 31°C.

The sequence of the cytoplasmic states in variety 9 is not an absolute series. It is relative to the environmental conditions (e.g. O_2 content) of the culture routine employed. This can be seen from the case of the rarely occurring type EL which can be transformed directly to type HT at 31°C and vice versa. In this respect the variety 9 situation bridges the gap between the different situations found in varieties 1 and 4.

There is also a difference in the serological properties of variety 1 and variety 4. In variety 4 the genetically corresponding types also correspond serologically. In variety 1 the antigenic types determined by the different alleles of a locus fall serologically into a series of distinct sub-groups (Beale 1954). It has been found that in variety 9 the antigenic types likewise are very distinct and can be sub-grouped to five G and seven HT types. In variety 1 and 9, therefore, it can be shown that both allelomorphs of a heterozygote are expressed (though with a slightly decreased immobilization reaction) and there is no display of dominance in any combination.

To sum up: variety 1 and 9 are similar in showing the existence of the temperature sequence and by the nature of the serological reactions, but they can be distinguished by the different temperature

stability properties of the alleles determining the antigenic specificities.

Because of the inherent similarity of varieties 1 and 9 it has been possible to compare systematically their antigenic types. The comparison has shown that the G types of each variety cross-react, although not exactly reciprocally or with any one to one correspondence of the sub-types. The antigenic types (D of variety 1 and HT of variety 9) allowed expression by the next cytoplasmic state in the temperature series showed no serological similarity. This possibly indicates that the cytoplasmic system in each variety is different and that the genes determining the D and HT types are not homologous.

The work of Landsteiner and others (reviewed, 1946), has shown that serological cross-reactions are of two types. In the first case the serological reactions parallel the taxonomic relations, with little or no intra-species variation. Substances immunologically reactive in this manner are serum proteins, snake venoms, toxins, enzymes, etc. In the second type marked intra-specific variation is found and in addition cross-reactions occur revealing apparently similar relationships between widely separate taxonomic units. For instance the A blood type of man is distinct from the other blood types,

yet it has properties very similar to the Forssman antigen found in various micro-organisms, plants, and animals. Cellular antigens in general fall into this class and the ciliary antigens of paramecia are not an exception. Cellular antigens are considered to be complex, consisting of combined protein and hapten. The frequent discovery of cross-reactions is considered as due to the chance occurrence of similar groupings of the components of the chemically simpler hapten portion of the antigen, rather than variation of the chemically more complex and immunologically more uniform protein part. Therefore, although antigens may be primary gene products or not far removed from them, in general it cannot be assumed that the serological detection of a similar antigen in widely distinct organisms implies that their specificity is determined by a homologous gene.

However in the case of the comparison of the varieties of P. aurelia, it seems valid to assume that serological similarity reflects genetic homology. The grounds for this assumption are that the cross-reactions which are observed between the antigenic types of the two varieties are limited to a group of types in each variety which come under the control of one gene. That is, the antigenic types controlled by one gene do not cross-react serologically

with types in the same variety determined by a different gene, and likewise the types with which they cross-react in another variety are controlled by a single gene. Thus in each individual variety the serological relations parallel the genetic relations, and serological cross-reactions occur between antigenic groups which are genetic units. For this reason the homology of the G determining gene in variety 1 and variety 9 is assumed. The homology of the gene loci is thus established but there is no apparent homology of the allelomorphs. Different mutant alleles may have become established in each sexually isolated variety, or, alternatively as the cytoplasmic systems of the two varieties are known to differ, differences in the corresponding cytoplasmic states may have altered the expression of identical alleles. At any rate variety 1 and variety 9 have diverged somewhat in their genetic constitution.

In variety 9 two independent multiple allele systems have been found. Recently some multiple-allele systems in other organisms - Aspergillus, Neurospora, maize, and Drosophila, - have been found to be composed of factors in pseudoallelic relationship. That the factors are pseudoalleles rather than true alleles has been realized by the discovery of rare instances of crossing-over within what was

considered one locus. In some cases the recombination of pseudoallelic factors may be very frequent. For instance, a recombination frequency of 0.1% has been recorded for certain pseudoallelic mutants of a biotin locus in A. nidulans. (Roper 1953).

In one of the multiple allele systems of variety 9 an attempt has been made to detect instances of intra-locus cross-over. None were found in the 7542 clones examined. This result however is subject to the following qualification. The basic assumption underlying the experiment was that as a result of crossing-over between factors determining two distinctive antigenic types, a recombinant factor would arise which would either determine an antigenic type with the combined characteristics of the parents, or determine a new antigenic type - possibly corresponding to one of the types already present in the other stocks. The method of screening the clones would not detect any other possible result of crossing-over, as for instance a reduced or enhanced reaction to the sera employed. The result of the experiment really indicates that a new serologically specific antigenic phenotype does not arise by a mechanism of intra-locus crossing-over above a frequency of 1 in 7500, if they arise by that mechanism at all. The phenotypic result of crossing-over may be of a

different order from that assumed, and the origin of the serologically distinct types of the multiple allele series may be mutational.

On more general grounds of comparison with other multiple allele-cell antigen systems, there is no reason to conclude that the genetic units are pseudoalleles and not true alleles. In B blood group system of cattle about 100 alleles have been recognised. The serum against a particular antigenic type is polyvalent and in the B system there are twenty-four serum factors. If the situation is interpreted by a system of closely linked loci there must logically be twenty four (Stormont 1955). However in the B blood group system there is no approach to the randomness of combination expected among the blood factors, supposing that there are multiple loci involved in their determination (Wright 1953). Instead some blood factors are found always dependent on one another, while others are independent. The heritable combinations of the known Rh blood factors of man are distributed in an analogous manner according to the interpretation of Stormont (1955). The existence of serological complexity is not therefore a valid ground for supposing the existence of 'pseudoallelic' genic elements. It must be remembered that antibodies produced by an immune response are not specific in

an absolute sense and therefore separable antigens cannot be assumed. The conclusion that is drawn from the blood group situation of cattle is that the antigenic types are determined by true alleles and not by the arrangement of closely linked loci (Stormont 1955). Therefore crossing-over within such a gene, if it can occur would be expected to be rare, and would produce a large effect on the phenotype, presumably by altering the chemical structure of the gene in the manner of a point mutation instead of by the assortment of sub-units. Consequently the recombinant types would in effect by identical to mutations and possibly as infrequent.

The serology of P. aurelia has not been studied using fractionation methods, but the negative result from the variety 9 cross-over experiment is in line with the above reasoning.

One of the most striking features of the biology of P. aurelia is the high degree of polymorphism of the antigenic characters. In variety 9 the same G and HT types are found in widely separate geographic regions, while in the one habitat - Blackford Pond - at least three G and three HT types have been shown to be present. There is no correlation between the occurrence of a particular G type and any of the HT types; they are associated randomly. Some types are more frequent than others,

both in geographical distribution and within one habitat.

It is likely that each isolated body of water supports a population having a particular spectrum of antigenic types. However this will be difficult to demonstrate thoroughly, since in the case of Blackford Pond one G type and one HT appeared only once among eighty-two organisms. Certain antigenic types therefore exist at frequencies low enough to make their detection difficult and it may be that all the possible types are present in any ecological unit. The numbers of variety 9 in one area have been found to fluctuate cyclically over the course of a year and it is very likely that there are differences in the maximum and minimum size of the population from year to year. Consequently the frequency of a particular antigenic type will vary and the proportions of the types found may alter, so that it is unlikely that a particular geographic region can be characterized either by the presence of specific antigenic types or by their frequencies.

Antigenic polymorphism is a feature of many cellular systems and acellular organisms. The specific serological types of the blood cell of man, cattle, mice, and other vertebrates, are well known. Bacteria, phage, and viruses also show considerable antigenic diversity. For instance the 'A' strain of

influenza virus displays a group specific antigen which conveys the 'A' type character and also type specific antigens peculiar to certain groups of strains. Different types occur in epidemics during the same periods and different types or asscciations of types appear in different years (Hilleman 1955). Antigenic diversity in parasitic organisms like viruses is obviously a selective advantage in that it is a means of annulling the consequences of the immune response of the individuals of the host population.

Huxley (1955) has introduced the terms Morphs and Morphisms to describe genetic polymorphic variation - i.e. 'sharply distinct genetic variants coexisting in temporary or permanent balance within an interbreeding population in a single spatial region'. The blood groups of man are a good instance of morphism. Different morph ratios exist in different enthic groups. The continued existence of a number of alternative morphic genes must involve some selective balance. The blood groups of man were earlier held to have no selective value, but recently differential selective values have been recognised with respect to viability, fertility, and susceptibility to various specific diseases (reviewed Huxley 1955). The selective advantages and disadvantages of various ambivalent

morphs tend to cancel one another out so that morph ratios in a given population are more or less stable.

The antigenic diversity of variety 9 of P. aurelia is a similar instance of species morphism. The selective mechanism which maintains the different morphic types in this example is not clear. In the laboratory, stocks of variety 9 have been adapted to tolerance of 1/1000N As_2O_3 solutions from the normal tolerance level of 1/2000N solution. This adaptation was non-genetic, being lost after one or two divisions in arsenic free medium. The antigenic types of these organisms did not change on adaptation. Similarly differences in the basic level of tolerance in the different strains (of different antigenic type) could not be distinguished. The antigenic constitution is in this case of no account in resisting the effects of the toxic agent. Paramecia live in an environment changing cyclically throughout the year and it may be that different morphs have a selective advantage at different times of the year and consequently this ambivalence of selective advantage would ensure that a number of morphs persisted in the population. It would be interesting to observe in parallel a number of discrete populations in succeeding years to observe the fluctuations in numbers and predominance of the antigens present.

The ecology of the paramecium fauna of one

habitat has been studied. As encysted or resistant stages of paramecia are not known, the populations in succeeding years must be reconstituted from the free-swimming survivors of the population of the previous year. Consequently considerable fluctuations in density and genetic composition are to be expected.

The fluctuation of the species density has been described. It will be noted that the optimal density of each species occurs at different times. In the case of the P. aurelia of variety 9 the cyclical change cannot depend primarily on the temperature. The outburst of variety 9 happens at a time when the temperature is high enough to allow rapid division but its course and timing does not correspond to the seasonal rise and fall of temperature. No noticeable variation in the pH of the water, which remained about pH 8.0, was found. The density of the population is probably a function of food, nutrients, and competition for microhabitats.

The study of the genetically marked antigenic characters has shown that (a) interbreeding takes place, (b) there is a considerable degree of polymorphism, and (c) the genetic content of the population changes during the course of the season. Further the behaviour of the allele frequencies of the two genes studied is different. The G antigenic type is probably the type expressed in the

pond environment, but this is not known definitely since few animals have been tested when collected. (Animals from the pond either die in antiserum solution and must be grown in laboratory medium for twelve hours before specific reactions can be shown, or they are at such low density in the collection tube that they cannot be found without incubation of the tubes. In one case however G type animals were isolated from a tube immediately after collection). The cause of the change of frequency of the HT antigenic type cannot be decided on the available evidence. Drift and mixing of populations or direct and indirect natural selection are all possibilities.

The situation is complicated by the effect the seasonal variation in temperature will have on the division rate of the paramecia. In the laboratory the rate is approximately once in twelve hours at 18°C and once in two weeks at 5°C. The maximum water temperature of Blackford Pond is about 20°C and the water temperature drops to 5°C by the beginning of November, so the population after passing through a period of rapid multiplication must enter a static phase for most of the year. Mating and conjugation presumably occurs only once a year at the time of peak density, and the heterozygotes detected in the population after this period are individuals of clones which have undergone conjugation some time previously and have-

still to go through autogamy.

Study of the composition of the population in succeeding years will perhaps indicate how much of the trend in change of antigenic type is due to the blending of different floating populations, and how much to forces of natural selection.

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7. SUMMARY.

The work described in this thesis has concerned two species of paramecia, P. aurelia and P. caudatum. Because of the technical difficulties involved in the crossing of P. caudatum a genetical study of the species was not possible and the work on P. caudatum has mainly concerned the breeding structure of the species. The work on the species P. aurelia has concerned the inheritance and the distribution of the antigenic variation of a newly discovered variety - variety 9.

The results of the work with P. caudatum can be summarized as follows:-

(1) Thirty-five stocks were collected from different localities. All these stocks could conjugate with one another. Varying amounts of F1 inviability were encountered in the crossing of these stocks.

Eighteen reactive stocks were collected from Blackford Pond. Intercrossing of these stocks gave progeny of high viability, but out-crossing of

these stocks to others of different geographical origin gave varying results from complete to non-viability of the F1.

(2) The cytology of the conjugation of stocks 1A and 24, which produced an F1 of low viability, is described. Abnormalities are first observed at the time of differentiation of the new anlagen. The anlagen may be abnormal in either form or number. Animals with macronuclear abnormalities rarely survive longer than the first two post-conjugation fissions.

(3) Two complementary mating types are found in the stocks of P. caudatum studied. After conjugation 13 of 14 marked clones possessed the same mating type as their cytoplasmic parent, which may therefore indicate that the inheritance of mating type is under cytoplasmic control.

In six stocks changes of mating type were observed in mass cultures. The changes all occurred in the one direction, type I changing to type II. The behaviour of one selfing clone from the F1 of a cross of stocks X31 and 28 is described and the possibility of its behaviour being similar to the switch of mating type in the mass cultures is discussed.

(4) In the stocks studied autogamy has not been found during six months observation, nor was the

phenomenon of ageing encountered in these stocks during the same period.

(5) Each stock of P. caudatum can manifest more than one antigenic type. No temperature series in the expression of these intra-stock antigenic types is observed, but in stock 24 a transformation series induced by immobilization with homologous serum has been found.

Some of the antigenic types found in different stocks are serologically similar. Also the anti-90G serum of P. aurelia (variety 1) immobilizes an antigenic type found in three stocks of P. caudatum; antisera against these types of P. caudatum reciprocally immobilize P. aurelia of type 90G.

(6) In conclusion the experimental results are discussed and it is proposed that the breeding system of P. caudatum is a continuously varying system rather than a system of discrete'varieties'. A system of mating compatibility is suggested such that genotypically similar stocks are more able to produce viable offspring than genotypically dissimilar ones, and that such a system has arisen as a result of geographical divergence. The mechanism of incompatibility is suggested as a nucleo-cytoplasmic incompatibility, but other explanations cannot be excluded.

The results of the work on the antigenic variation of variety 9 of *P. aurelia* can be summarized as follows:-

(1) Each stock can manifest more than one antigenic type. In all the stocks of variety 9 collected two antigenic types are normally found, a G type at temperatures between 14°C and 28°C, and an HT type at temperatures between 31°C and 33°C.

(2) The G antigenic types of different stocks fall serologically into five sub-groups, and the HT types into seven sub-groups. No cross-reactions are found between the G and HT antigenic types.

(3) The G antigenic types are determined by the multiple alleles of one gene. The HT antigenic types are determined by a multiple allele series of another gene. The loci are not linked. The particular gene expressed depends on the cytoplasm. Two states of the cytoplasm are normally found, one at temperatures up to 28°C, and the other between temperatures of 31°C and 33°C.

(4) The antigenic types determined by the multiple alleles are stable in inheritance. No unexpected types were found in 7500 exautogamous clones examined after a cross of types 509G and 510G.

(5) Serological comparison of variety 1 and

variety 9 shows that the antigens of each are distinct with no one to one correspondence. Cross-reactions between the G antigenic types of varieties 1 and 9 occur, but cross-reactions between the D types of variety 1 and the HT types of variety 9 are not found.

(6) The geographical distribution of the different antigenic types of variety 9 is discussed. Some antigenictypes are more common than others but no geographical limitation of any of the types is likely. Stocks from widely distinct localities may carry the same antigen. The different G and HT types do not occur in particular associations.

(7) In the study of a population in one habitat, three G types and three HT types have been found. The population densityin this habitat (Blackford Pond) shows a cyclical change during the course of one year. At the same time a change in the proportions of the two predominant HT types is observed, while the proportions of the G types show no definite trend. The detection of heterozygotes of the g gene indicates that variety 9 is a functional breeding unit in the wild.

(8) In conclusion the genetical system of the inheritance of the antigens of variety 9 is outlined and compared to the systems already known in varieties 1 and 4, and the question of whether the different antigenic types of variety 9 are controlled by multiple alleles or pseudoalleles is

discussed. Evidence has also been given for the homology of the genes controlling the G antigenic types of varieties 1 and 9.

The antigenic variation in stocks of different geographical origin and in stocks from the same locality is compared, and the fluctuation in the numbers of paramecia found in Blackford Pond and its effect on the antigenic composition of the population is discussed.

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